

[Designation of Document] Specification

[Title of the Invention] HUMAN Nap1 PROTEIN

[Claims]

[Claim 1] A human Nap1 protein having the amino acid sequence represented by SEQ ID NO. 2 or a protein having an amino acid sequence which includes substitution, deletion or addition of one or more amino acids of the amino acid sequence represented by SEQ ID NO. 2 and having apoptosis-suppressing activities.

[Claim 2] A DNA encoding the protein according to Claim 1.

[Claim 3] A DNA according to Claim 2, having the nucleotide sequence represented by SEQ ID NO. 1.

[Claim 4] A DNA capable of hybridizing to the nucleotide sequence of the DNA according to Claim 2 or 3 under stringent conditions and encoding a protein having apoptosis-suppressing activities.

[Claim 5] A recombinant vector containing the DNA according to any one of Claims 2 to 4 and a vector.

[Claim 6] A transformant which is obtained by introducing the vector according to Claim 5 into a host cell.

[Claim 7] A process for producing the said protein, which comprises culturing the transformant according to Claim 6 in a medium to produce and accumulate the protein according to Claim 1 in the culture and harvesting the protein from the culture to be obtained.

[Claim 8] A therapeutic composition for Alzheimer's disease containing the protein according to Claim 1 as an active ingredient.

[Claim 9] An oligonucleotide having a continuous 5 to 60-bp nucleotide sequence out of the DNA nucleotide sequences according to any one of Claims 2 to 4 or an oligonucleotide having a sequence complementary to said oligonucleotide.

[Claim 10] An oligonucleotide according to Claim 9, which has a nucleotide sequence represented by SEQ ID NO. 18 or 19.

[Claim 11] An oligonucleotide according to Claim 9, which has a nucleotide sequence represented by SEQ ID NO. 27 or 29.

[Claim 12] A method for detecting an mRNA of a human Nap1 gene using the oligonucleotide according to Claim 9 or 10.

[Claim 13] A diagnostic reagent for Alzheimer's disease containing the oligonucleotide according to Claim 9 or 10.

[Claim 14] A method for repressing transcription of the human Nap1 gene or translation of mRNA thereof using the oligonucleotide according to Claim 9 or 11.

[Claim 15] A therapeutic composition for apoptosis-participating diseases containing the oligonucleotide according to Claim 9 or 11.

[Claim 16] An antibody recognizing the protein according to Claim 1.

[Claim 17] An immunoassay of the protein according to Claim 1 using the antibody according to Claim 16.

[Claim 18] A diagnostic reagent for Alzheimer's disease containing the antibody according to Claim 16.

[Claim 19] A therapeutic composition for apoptosis-participating diseases containing the antibody according to Claim 16 as an active ingredient.

[Detailed Description of the Invention]

[Field of the Invention]

This invention relates to a human Nap1 protein, a DNA encoding said protein, a process for producing said protein, an antibody recognizing said protein and the use of said protein.

[Prior Art]

Alzheimer's disease is a progressive neurodegenerative disorder of the brain, which is one of the causes of senile dementia. The Alzheimer's disease is divided into familial one considered to be caused by genetic mutation and sporadic one, and the latter accounts for the majority. Its pathological features in both the familial case and the sporadic case are cerebral atrophy caused by neuronal loss and massive neuronal cell death, neuronal degeneration called neurofibrillary tangles, amyloid fibril deposition in the intercellular spaces called senile plaque, etc. Although many studies have been made of factors participating in the onset of said disease, the biochemical mechanism leading to these disorders has not yet been elucidated well.

Familial Alzheimer's disease is considered to be caused by genetic mutation. And, from the genetic analysis of the familial Alzheimer's disease, β -amyloid precursor protein [*Nature*, 325, 733 (1987)], presenilin 1 [*Nature*, 375, 754 (1995) and presenilin 2 [*Science*, 269, 973 (1995)] have been reported as Alzheimer's disease-related genes. Besides, as a gene considered to be a risk factor of the onset, the type 4 allele out of allele polymorphisms of apolipoprotein E (apo E) [*Science*, 261, 921 (1993)] have been reported.

Although experiments using normal cells have brought a report that apoptosis was induced when a mutated β -amyloid precursor protein gene was introduced and expressed in a cell [*Science*, 272, 1349 (1996)], a report that cell death was induced when β -amyloid peptide derived from β -amyloid precursor protein was administered to neuronal cells [*Science*, 245, 417 (1989)], a report that apoptosis was induced by a peptide corresponding to a part of β -amyloid peptide [*Proceedings of the National Academy of Sciences of the United States of America*, 90, 7951 (1993)], there are still unclear points about how the above gene participates in neuronal cell death and the onset of Alzheimer's disease.

Over against this, it has been known that apoptosis occurs in neuronal cells of a patient with Alzheimer's disease [*Experimental Neurology*, 133, 225 (1995)]. If such apoptosis in neuronal cells is brought by the same mechanism as that in which β -amyloid precursor protein or the like participates, it is considered that the neuronal cell death is repressed by repressing apoptosis to make the prevention and the treatment of Alzheimer's disease possible.

On the other hand, sporadic Alzheimer's disease accounts for the majority of Alzheimer's disease, the onset mechanism of which has scarcely been known. It is not also unclear whether the above mechanism of neuronal cell death with which the above gene associates also participates in the sporadic Alzheimer's disease or not.

By the way, an Nck associated protein (hereinafter abbreviated to Nap) is a protein which was identified as a 125-kDa protein in a bovine brain extract and which specifically but indirectly associates with the 1st SH3 domain of an intracellular signal transduction molecule Nck [*Biochemical and Biophysical Research Communication*, 219, 509 (1996)].

Before now, a mouse Nap1 and a cDNA encoding a rat Nap1 has been isolated. That is, based on an inference from the partial amino acid sequence of a Nap1 protein purified from a bovine brain, the mouse cerebellum cDNA clone H19 [*European Journal of Neuroscience*, 2, 704 (1990)] is considered to be a mouse Nap1 partial cDNA clone, then a rat brain cDNA library was screened using the cDNA of said H19 as a probe. As a result of it, a full length rat Nap1 cDNA was obtained. This cDNA encoded rat Nap1 protein comprising 1128 amino acids [*Biochem. Biophys. Res. Commun.*, 219, 509 (1996)].

Incidentally, as proteins having homology to rat Nap1, gene expression products of human Hem-1 cDNA cloned as a gene which is specifically expressing in a human hemocyte [*Biochimica et Biophysica Acta*, 1090, 241 (1991)], a Drosophila gene dhem-2 which is considered to play an important role in oogenesis and early embryogenesis [*Journal of Molecular Biology*, 251, 41 (1995)] and a rat Hem-2 cDNA obtained from a rat brain cDNA library using H19 cDNA as a probe [*J. Mol. Biol.*, 251, 41 (1995)] are known. The amino acid sequence homologies of respective proteins with the rat Nap1 are such high values as 59% in the human Hem-1, 60% in the Drosophila dhem-2 and 94% in the rat Hem-2.

The Nck with which Nap1 is associated is an intracellular protein comprising 377 amino acids and has an SH2 domain and three SH3 domains [*Nucleic Acids Research*, 18, 1048 (1990)]. Generally, it is considered that the SH2 domain has a property of binding to a domain containing phosphorylated tyrosine and the SH3 domain has a property of binding to a proline-rich domain, and molecules having these domains are considered to participate in intracellular signal transduction. That is, a large number of growth factor receptors intracellularly carry a domain having tyrosine kinase activities, and it is considered that the tyrosine kinase is activated by binding to a ligand to phosphorylate tyrosine residues of own or other molecules to thereby start the signal transduction. For example, it is reported that Nck, via its SH2 domain, binds to an autophosphorylated tyrosine of a tyrosine kinase type receptor of a cell growth factor such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) or the like to undergo phosphorylation [*Proc. Natl. Acad. Sci. USA*, 89, 8894 (1992); *Molecular & Cellular Biology*, 12, 5824 (1992); *Journal of Biological Chemistry*, 270, 6729 (1995); *Biochemistry & Molecular Biology International*, 36, 465 (1995)].

As molecules binding to the Nck SH3 domain, PAK (p21ras-activated kinase), p21ras activation factor Sos by guanine nucleotide exchange, NAK (Nck-associated kinase) as a

serine/threonine kinase, c-cbl as a proto-oncogene to be phosphorylated upon simulation of various receptors and SAKAP II (Src A box Nck-associated protein II) as a causative gene of Whiskoff-Aldrich syndrome have been reported [*J. Biol. Chem.*, 270, 22731 (1995); *Mol. Cell. Biol.*, 15, 1169 (1995); *J. Biol. Chem.*, 270, 7359 (1995), *J. Biol. Chem.*, 269, 17363 (1994); and *Mol. Cell. Biol.*, 15, 5725 (1995)].

Judging from such associated molecules as above, it is considered that Nck is playing an important role mainly in mitogenic signal transduction pathways of growth factors, oncogenes, etc. Actually, it is reported that, when the Nck was overexpressed in NIH3T3 cells or 3Y1 cells as fibroblast cells, said cells are oncogenically transformed [*Mol. Cell. Biol.*, 12, 5824 (1992) and *Mol. Cell. Biol.*, 12, 5834 (1992)]. Besides, PC12 cells are differentiated into a neuronal cells, arrested the growth and made to project neurites by a nerve-derived growth factor (NGF). It is reported that, as a result of overexpressing the Nck in PC12 cells, the differentiation was blocked and the proliferation was continued even by administering the NGF to the cells [*Oncogene*, 12, 2351 (1996)]. Furthermore, there also is a report that Nck activated the promoter of transcription factor fos as a proto-oncogene [*Mol. Cell. Biol.*, 15, 1169 (1995)].

Therefore, it is considered that Nap1 which is a molecule specifically associated with the Nck SH3 domain also plays a role in the cell proliferation system.

However, its direct function and action are unclear. In addition, there is neither a report showing the relation between Nap1 and Alzheimer's disease nor a report that Nap1 has an inhibitory effect on apoptosis. Furthermore, with respect to the information about human Nap1 gene and protein, only some EST (Expressed Sequence Tag; collection of information about 3'-end and 5'-end nucleotide sequences of a large number of cDNA clones in a cDNA library with redundancy), that is nucleotide sequences of the 5'-end moieties of human cDNA clones, which show homology to rat Hem-2 or mouse H19, are reported in nucleotide sequence data base Genbank, its whole structure is still unknown.

Therefore, the elucidation of genes and the like related to Alzheimer's disease is anticipated for the diagnosis, the prevention and the treatment of Alzheimer's disease.

[Problem to be solved by the Invention]

The present invention purposes to provide a human Nap1 protein, a DNA encoding said protein, a process for producing said protein, an antibody recognizing said protein and the use of said protein.

[Means to Solve the Problems]

The present inventors, as a result of making intensive studies for solving the above problems, succeeded in isolating human Nap1 (Nck associated protein 1) related to Alzheimer's disease and cloning a DNA encoding said protein, thereby completing the present invention.

That is, the present invention relates to the human Nap1 protein, DNA encoding the

protein, a process for obtaining the DNA, a recombinant vector comprising the DNA and a vector, a transformant obtained by introducing the recombinant vector into a host, a process for producing the human Nap1 protein encoded by the DNA using the transformant, an antibody recognizing the protein, a reagent and a process for diagnosing Alzheimer's disease comprising the protein, DNA and antibody, and a therapeutic composition for Alzheimer's disease comprising the protein, the use of the DNA or antibody in diagnosis, prognosis or treatment for apoptosis-participating diseases.

[Embodiments of the Invention]

The protein according to the present invention includes a human Nap1 protein having the amino acid sequence represented by SEQ ID NO. 2 or a protein having an amino acid sequence which includes substitution, deletion or addition of one or more amino acids of the amino acid sequence represented by SEQ ID NO. 2 and having apoptosis-suppressing activities.

Among proteins of the present invention, a protein comprising an amino acid sequence containing one or several deleted, substituted or added amino acids and having apoptosis suppression activities can be prepared according to the methods described in *Nucl. Acids Res.*, 10, 6487 (1982); *Proc. Natl. Acad. Sci. USA*, 79, 6409 (1982); *Proc. Natl. Acad. Sci. USA*, 81, 5662 (1984); *Science* 224, 1431 (1984); PCT WO 85/00817 (1985); *Nature*, 316, 601 (1985); *Gene*, 34, 315 (1985); *Nucl. Acids Res.*, 13, 4431 (1985); Current Protocols in Molecular Biology, Chapter 8, "Mutagenesis of Cloned DNA", John Wiley & Sons, Inc. (1989) etc.

The DNA according to the present invention includes a DNA encoding the protein according to the present invention, a DNA having the nucleotide sequence represented by SEQ ID NO. 1, and a DNA capable of hybridizing with the nucleotide sequence of the DNA under stringent conditions.

'A DNA capable of hybridizing under stringent conditions' described above means a DNA which is obtained by using the DNA having the nucleotide sequence represented by SEQ ID NO. 1 as a probe and employing colony hybridization method, plaque hybridization techniques, Southern blot hybridization techniques or the like. Specifically, a DNA obtained by carrying out hybridization at 65°C in the presence of 0.7M to 1.0M NaCl using a filter on which a colony- or plaque-derived DNA is immobilized and subsequent identification by washing the filter at 65°C with a 0.1 x - 2 x SSC (saline sodium citrate) solution (1 x SSC solution comprises 150mM sodium chloride and 15mM sodium citrate; n x means the solution with n-fold concentration) can be given. The hybridization can be carried out in compliance with the methods described in experimented manuals such as Molecular Cloning: A Laboratory Manual, Second Edition [Sambrook, Fritsch, Maniatis Eds., Cold Spring Harbor Laboratory Press, 1989, hereinafter "Molecular Cloning Second Edition", etc]. As specific examples of the hybridizable DNA, DNAs having at least 60% or more, preferably 80% or more, more preferably 95% or more homology with the nucleotide sequence represented by SEQ ID NO. 1 can be enumerated.

In addition, the DNA of the present invention includes a DNA having a part of nucleotide sequence of the said DNA described above or a DNA having a sequence complementary to said DNA. As example of the DNA having a part of nucleotide sequence of the present DNA, an oligonucleotide having the identical nucleotide sequence as continuous 5 to 60 residues, preferably 10 to 40 residues of the nucleotide sequence represented by SEQ ID NO. 1 can be given, and, as an example of the DNA having a sequence complementary to the DNA having a part of nucleotide sequence of the present DNA, an antisense oligonucleotide of said oligonucleotide can be given. A DNA having a nucleotide sequence represented by SEQ ID NO. 18 can be given as an example of said oligonucleotide, and a DNA having a nucleotide sequence represented by SEQ ID NO. 27 and 29 can be given as an example of said antisense oligonucleotide.

Hereinafter the present invention will be described in detail. Incidentally, ordinary methods described in this text means those described in experimental manuals such as 'Molecular Cloning, Second Edition' and the like.

1. Preparation of DNA Relevant to Alzheimer's Disease

In order to elucidate the molecular mechanism of sporadic Alzheimer's disease, it is important to find out molecules participating in its onset. Exploration of a gene showing a different expression pattern between the brain of a sporadic Alzheimer's disease patient and that of a non-Alzheimer's disease person, that is, a gene showing quantitative change affords a clue to the discovery of molecules participating in the onset thereof. Then, taking notice of the change in expression of mRNA in the brain of a sporadic Alzheimer's disease patient and that of a non-Alzheimer's disease person, a DNA related to the onset of Alzheimer's disease is prepared employing the differential display technique [*Science*, 257, 967 (1992); *FEBS Letters*, 351, 231 (1994)].

That is, the total RNAs are prepared from the brain of a sporadic Alzheimer's disease patient and that of a non-Alzheimer's disease person first of all. As examples of the method for preparing the total RNA from brain tissues, the guanidine thiocyanate-caesium trifluoroacetate method [*Methods in Enzymology*, 154, 3 (1987)], the AGPC method [*Experimental Medicine*, 2 1937 (1991)], etc. can be given.

In addition, as examples of the method for preparing mRNA as a poly (A)+RNA from the total RNA, the oligo(dT) immobilized cellulose column technique (Molecular Cloning, Second Edition), etc. can be given.

Furthermore, it is also possible to prepare mRNA directly from brain tissues by using a kit such as Fast Track mRNA Isolation Kit (manufactured by Invitrogen), Quick Prep mRNA Purification Kit (manufactured by Pharmacia) or the like.

From each of the above RNAs extracted from brain tissues, cDNAs are synthesized using anchor primers according to ordinary methods, and subsequently each of these cDNAs are subjected to PCR using the anchor primers and arbitrary primers to thereby amplify the

cDNAs.

The anchor primer means a primer prepared by adding an oligonucleotide exclusive of thymidine such as adenine, guanine or cytosine to the 3'-end of an oligo(dT) sequence which hybridizes with the 3'-end polyA sequence of a mRNA, as examples of which oligonucleotides having the nucleotide sequences represented by SEQ ID NOS. 4 to 6 can be given.

The arbitrary primer means an oligonucleotide which can amplify against varieties of cDNA sequences, and can obtain a large number of cDNA amplification fragment by only one reaction, as examples of which OPA-1 to -20, OPB-1 to -20, OPC-1 to -20, OPD-1 to -20, etc. manufactured by Operon Technologies can be given. The length of an arbitrary primer is preferably on the order of 10 to 20 bases.

Subsequently, each of the above cDNAs amplified by PCR is electrophoresed on a polyacrylamide gel, and the quantity of amplification of each band can be determined by fluorescently staining each amplified cDNA with a DNA-specific fluorescent stain such as SYBR Green I or the like and measuring the intensity of fluorescence thereof using a fluorimeter. Besides, when an anchor primer previously labeled fluorescently at the 5' end is used in PCR, the intensity of fluorescence of a band can be determined just after the electrophoresis without using a fluorescent stain. The fluorescent label of the 5'-end of the primer can be carried out using fluorescein isothiocyanate according to an ordinary method.

The intensity of fluorescence of respective bands are compared, the gel corresponding the position of a band with a changed fluorescence intensity is cut out, the gel cut out is placed in a reaction solution to carry out PCR, and whereby a DNA fragment in the gel is amplified.

Said amplified DNA fragment is ligated into a vector according to an ordinary method as it is or after blunting its end with pfuDNA polymerase and then analyzed employing ordinary used nucleotide sequence analysis methods, for example, the dideoxy method of Sanger et al. [*Proc. Natl. Acad. Sci. USA*, 74, 5463 (1977)] or a nucleotide sequence analyzer such as a DNA Sequencer 373A (manufactured by Perkin Elmer) or the like, and whereby the nucleotide sequence of said DNA is determined.

As examples of the vector in which said amplified DNA fragment is ligated, pCR-Script Amp [manufactured by Stratagene; *Stratagies*, 5, 6265 (1992)], pT7Blue T-Vector (manufactured by Novagen), pCR2.1 [manufactured by Invitrogen; *Bio/Technology*, 9, 657 (1991)], pCR-TRAP (manufactured by GenHunter), pTA (manufactured by Nippon Gene K.K.), pNoTA T7 (manufactured by 5'→3', Inc.), etc. can be given.

The novelty of thus determined nucleotide sequence can be confirmed by searching nucleotide sequence data bases such as GenBank, EMBL, DDBJ, etc. using a homology-searching program such as BLAST or the like to find out the absence of a nucleotide sequence showing obvious homology with a nucleotide sequence in the data bases, as considered identical.

As examples of the DNA obtained as above, a DNA having the sequence represented

by SEQ ID NO. 3 and the like can be given.

In case that the DNA obtained according to the above method is a partial DNA fragment of the cDNA corresponding to the mRNA of the gene the expression of which is changed in the brain of a Alzheimer's disease patient with, a full length cDNA can be obtained according to the methods of (1), (2) or (3) set forth below.

(1) Utilization of cDNA library

By carrying out screening by means of hybridization with various cDNA libraries using the above DNA fragment as a probe, a full length cDNA can be obtained.

Hereinafter, a method for making a cDNA library will be described.

As examples of the method for making a cDNA library, methods described in Molecular Cloning, Second Edition; Current Protocols in Molecular Biology, Supplement 1 to 34, Greene Publishing Associates and Wiley-Interscience, 1987-1996 Edition (hereinafter abbreviated to Current Protocols in Molecular Biology, Supplement 1 to 34); etc. or methods employing commercially available kits, for example SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning (manufactured by Life Technologies) or ZAP-cDNA Synthesis Kit (manufactured by Stratagene) can be given. In addition, a commercially available article of the very cDNA library, a human brain cDNA library supplied by CLONETECH, etc. are also utilizable.

As a cloning vector for making a cDNA library, any of a phage vector, a plasmid vector, etc. can be used as far as it is able to replicate autonomously in the *Escherichia coli* strain K12. Specifically, ZAP Express [manufactured by Stratagene; *Strategies*, 5, 58 (1992)], pBluescript II SK(+) [*Nucleic Acids Research*, 17, 9494 (1989)], Lambda ZAP II (manufactured by Stratagene), λ gt10, λ gt11 [*DNA Cloning*, A Practical Approach, 1, 49 (1985)], λ TriplEx (manufactured By CLONETECH), λ ExCell (manufactured by Pharmacia), pT7T318U (manufactured by Pharmacia, Inc.), pcD2 [*Mol. Cell. Biol.*, 3, 280 (1983)], pUC18 [*Gene*, 33, 103 (1985)], etc. can be enumerated.

As a host microorganism, any microorganism can be used as far as it is a microorganism belonging to *Escherichia coli*. Specifically, *Escherichia coli* XL1-Blue MRF' [manufactured by Stratagene; *Strategies*, 5, 81 (1992)], *Escherichia coli* C600 [*Genetics*, 39, 440 (1954)], *Escherichia coli* Y1088 [*Science* 222, 778 (1983)], *Escherichia coli* Y1090 [*Science*, 222, 778 (1983)], *Escherichia coli* NM522 [*J. Mol. Biol.*, 166, 1 (1983)], *Escherichia coli* K802 [*J. Mol. Biol.*, 16, 118 (1966)], *Escherichia coli* JM105 [*Gene*, 38, 275 (1985)], etc. are used.

The selection of a cDNA clone from the cDNA library can be carried out according to colony hybridization method or plaque hybridization method using an isotope- or a fluorescence-labeled probe (Molecular Cloning, Second Edition).

The aimed DNA can be obtained from the selected clone according to a generally known method.

(2) A cDNA is synthesized from a mRNA according to the above method, adapters are added to the both end of said cDNA, and a 5'-end cDNA and a 3'-end cDNA can be obtained respectively from the amplification fragment by 5'-RACE (rapid amplification of cDNA ends) and 3'-RACE [Proc. Natl. Acad. Sci. USA, 85, 8998 (1988)] according to which PCR is carried out using primers based on the nucleotide sequences of said adapters and that of the amplification fragment. And, the aimed DNA can be obtained by ligating the obtained cDNA to the amplification fragment.

(3) The aimed DNA can be obtained by PCR (PCR Protocols, Academic Press, 1990) using primers prepared based on the nucleotide sequences of the 5'-end and 3'-end of the full length cDNA obtained according to the method of (1) or (2) and a cDNA synthesized from a mRNA or a cDNA library as a template.

The nucleotide sequences of the DNAs obtained according to the above methods can be determined by the above nucleotide sequencing method. Also the novelty of said sequences can be confirmed according to the above method. As examples of the DNA having a novel nucleotide sequence confirmed according to the above method, a DNA having the sequence represented by SEQ ID NO. 1 and the like can be given. In addition, the amino acid sequence of the human Nap1 protein according to the present invention is given as an example in SEQ ID NO. 2, in which variations such as deletion, substitution, addition and the like of one or more (preferably one or several) amino acids may occur in said amino acid sequence as far as a protein comprising this amino acid sequence has apoptosis-suppressing activities. For example, a protein having a sequence which has the deletion of the 1st methionine of the amino acid sequence represented by SEQ ID NO. 2 is also included in the protein according to the present invention as far as it has apoptosis-suppressing activities.

Yet, the introduction of a mutation is carried out by the conventional means or by using a commercially available kit (for example, Site-Directed Mutagenesis Kit from CLONETECH).

It is also possible to prepare the aimed DNA by carrying out chemical synthesis using a DNA synthesizer on the basis of the determined DNA nucleotide sequence. As examples of the DNA synthesizer, a thiophosphite method-applying DNA synthesizer (manufactured by Shimadzu Corporation), a phosphor amidite method-applied DNA synthesizer model 392 (manufactured by Perkin-Elmer), etc. can be given.

An oligonucleotide having a partial sequence of a DNA participating in apoptosis and an antisense oligonucleotide can be obtained by using said DNA and DNA fragment according to an generally known method or using a DNA synthesizer.

As examples of said oligonucleotide or antisense oligonucleotide, a sense primer corresponding to the 5'-end nucleotide sequence, an antisense primer corresponding to the 3'-end nucleotide sequence of the part of nucleotide sequence of a mRNA for detection. However, a base corresponding to uracyl in the mRNA is thymidine in the oligonucleotide primer.

As the sense primer and the antisense primer, oligonucleotides with 10 to 40 residues whose respective melting temperatures (T_m) and number of bases are not different extremely are preferable.

As examples of the oligonucleotide or the antisense oligonucleotide having a part of sequence of the human Nap1 DNA, nucleotides having the nucleotide sequence represented by SEQ ID NO. 18, 19 or 27, 29 can be given.

In addition, also a derivative of said nucleotide can be used in the present invention, as examples of which a methyl-modified one and a phosphorothioate-modified one of said nucleotide can be enumerated.

2. Preparation of Human Nap1 Protein

In order to express the human Nap1 DNA obtained according to the method described in 1 above in a host cell, methods described in Molecular Cloning, Second Edition; Current Protocols in Molecular Biology, Supplement 1 to 34; etc. can be adopted. That is, a transformant expressing the protein of the present invention can be obtained by inserting a DNA encoding the protein of the present invention downstream of a promoter in an appropriate expression vector to construct a recombinant vector and then introducing the same into a host cell.

As the host cell, bacteria, yeast, animal cells, insect cells, etc. can be used as far as they can express the aimed gene.

As the expression vector, a vector which is able to replicate autonomously or is capable of being integrated into the chromosome in the above host cell and which contains a promoter at a site where the protein of the present invention can be transcribed is used.

In case of using a prokaryotic cell such as a bacterium or the like as a host cell, it is preferred that the human Nap1 expression vector should be able to replicate autonomously in the prokaryotic cell and simultaneously be composed of a promoter, a ribosome binding sequence, a human Nap1 gene and a transcription termination sequence. In addition, a gene controlling the promoter may be contained.

As examples of the expression vector, pKK233-2 (manufactured by Pharmacia), pSE280 (manufactured by Invitrogen), pGEMEX-1 (manufactured by Promega), pQE-8 (manufactured by QIAGEN), pKYP10 (Japanese Patent Application Laid open No. 110,600/1983), pKYP200 [*Agricultural Biological Chemistry*, **48**, 669 (1984)], pLSA1 [*Agric. Biol. Chem.*, **53**, 277 (1989)], pGEL1 [*Proc. Natl. Acad. Sci. USA*, **82**, 4306 (1985)], pBluescript II SK(-) (manufactured by Stratagene), pTrs30 [prepared from *Escherichia coli* JM109/pTrs30 (FERM BP-5407), pTrs 32 [prepared from *Escherichia coli* JM 109/pTrs 32 (FERM BP-5408)], pGKA2 [prepared from *Escherichia coli* IGKA (FERM BP-6798), see Japanese Patent Application Laid open No. 221,091/1985], pTerm2 (see Japanese Patent Application Laid open No. 22,979/1991, US4686191, US4939094, US5160735), pGEX (manufactured by Pharmacia), pET System (manufactured by Novagen), pSupex, etc. can be given.

As the promoter, any promoter may be employed as far as it can be expressed in a host cell such as *Escherichia coli* or the like, as examples of which promoters originated from *Escherichia coli*, phages, etc. such as *trp* promoter (*P_{trp}*), *lac* promoter, *P_L* promoter, *P_R* promoter, T7 promoter, etc. can be given. In addition, artificially designed or modified promoters such as a promoter composed of two *P_{trp}* connected in series (*P_{trp}* × 2), *tac* promoter, *lacT7* promoter, *letI* promoter, etc. can be used also.

As the ribosome binding sequence, a plasmid having a suitably regulated spacing (e.g., 6 to 18 bases) between the Shine-Dalgarno sequence and the starting codon is preferably used.

In the present recombinant vector, it is preferable that a transcription termination sequence is positioned just downstream from the structural gene though said sequence is not always necessary for the expression of the present DNA.

As a host cell, microorganisms belonging to the genera *Escherichia*, *Serratia*, *Corynebacterium*, *Brevibacterium*, *Pseudomonas*, *Bacillus*, etc., for example, *Escherichia coli* XL1-Blue, *Escherichia coli* XL2-Blue, *Escherichia coli* DH1, *Escherichia coli* MC1000, *Escherichia coli* KY3276, *Escherichia coli* W1485, *Escherichia coli* JM109, *Escherichia coli* HB101, *Escherichia coli* No. 49, *Escherichia coli* W3110, *Escherichia coli* NY49, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Brevibacterium immariophilum* ATCC14068, *Brevibacterium saccharolyticum* ATCC14066, *Brevibacterium flavum* ATCC14067, *Brevibacterium lactofermentum* ATCC13869, *Corynebacterium glutamicum* ATCC13032, *Corynebacterium acetoacidophilum* ATCC13870, *Microbacterium ammoniaphilum* ATCC15354, etc. can be given.

As an introduction method of the recombinant vector, any method can be adopted as far as it is a method for introducing a DNA into the above host cell, as examples of which a method using calcium ions [*Proc. Natl. Acad. Sci. USA*, 69, 2110 (1972)], the protoplast method (Japanese Patent Application Laid open No. 248,394/1988), etc. can be given.

In case of using a yeast strain as a host cell, YEp13 (ATCC37115), Yep24 (ATCC37051), Ycp50 (ATCC37419), etc. can be used as examples of an expression vector.

As a promoter, any promoter may be used as far as it can be expressed in the yeast strain, as examples of which a promoter of a glycolytic gene such as hexokinase or the like, *gal 1* promoter, *gal 10* promoter, heat shock protein promoter, *MF α 1* promoter, *CUP 1* promoter, etc. can be given.

As a host cell, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Trichosporon pullulans*, *Schwanniomyces alluvius*, etc. can be enumerated.

As an introduction method of the recombinant vector, any method can be adopted as far as it is a method for introducing a DNA into yeast, as examples of which electroporation [*Methods. Enzymol.*, 194, 182 (1990)], spheroplast method [*Proc. Natl. Acad. Sci. USA*, 84, 1929 (1978)], lithium acetate method [*Journal of Bacteriology*, 153, 163 (1983)], etc. can be given.

As an expression vector in case of using an animal cell as a host cell, for example, pAGE107 [Japanese Patent Application Laid open No. 22,979/1991; *Cytotechnology*, 3, 133

(1990)], pAS3-3 (Japanese Patent Application Laid open No. 227,075/ 1990), pCDM8 [*Nature*, 329, 840 (1987)], pcDNAI/Amp (manufactured by Invitrogen), pREP4 (manufactured by Invitrogen), pAGE103 [*Journal of Biochemistry*, 101, 1307 (1987)], pAGE210, etc. are used.

As a promoter, any promoter can be used as far as it can be expressed in an animal cell, as examples of which IE (immediate early) gene promoter of cytomegalovirus (CMV), SV40 early promoter, metallothionein promoter, etc. can be given. In addition, human CMV IE gene enhancer may be used together with a promoter.

As a host cell, Namalwa cell which is a human cell, COS cell which is a monkey cell, CHO cell which is a Chinese hamster cell, HBT5637 (Japanese Patent Application Laid open No. 299/1988), etc. can be enumerated.

As an introduction method of the recombinant vector, any method can be adopted as far as it is a method for introducing a DNA into an animal cell, as examples of which electroporation [*Cytotechnology*, 3, 133 (1990)], calcium phosphate method (Japanese Patent Application Laid open NO. 227,075/1990)], lipofection method [*Proc. Natl. Acad. Sci. USA*, 84, 7413 (1987)], etc. can be given.

In case of using an insect cell as a host cell, a protein can be expressed according to the methods described, for example, in Baculovirus Expression Vectors, A Laboratory Manual; Current Protocols in Molecular Biology, Supplement 1 to 34; *Bio/Technology*, 6, 47 (1988); etc.

That is, after cotransfecting a recombinant gene-transfer vector and a baculovirus into an insect cell to obtain a recombinant virus in the supernatant of the insect cell culture, the insect cell is infected with the recombinant virus, and whereby a protein can be expressed.

As examples of the gene-transfer vector to be used in said method, pVL1392, pVL1393, pBlueBacIII (all manufactured by Invitrogen), etc. can be given.

As a baculovirus, a virus which infects an insect belonging to the *Noctuidae* family such as *Autographa californica* nuclear polyhedrosis virus and the like can be used.

As an insect cell, Sf9 and Sf21 [Baculovirus Expression Vectors, A Laboratory Manual, W.H. Freeman and Company, New York (1992)] which are ovarian cells of *Spodoptera frugiperda*, High5 (manufactured by Invitrogen) which is an ovarian cell of *Trichoplusia ni*, etc. can be used.

As a cotransfection method of the above recombinant gene-transfer vector and baculovirus into an insect cell, for example, calcium phosphate method (Japanese Patent Application Laid-open No. 227,075/1990), lipofection method [*Proc. Natl. Acad. Sci. USA*, 84, 7413 (1987)], etc. can be enumerated.

As an expression method of a gene, secretory production, fused protein expression, etc. can be carried out in accordance with the methods described in Molecular Cloning, Second Edition in addition to direct expression. When the expression is carried out using yeast, an animal cell or an insect cell, a glycosylated protein can be obtained.

A transformant obtained as above is cultured in a medium to make it produce and

accumulate the present protein in the culture and the protein is harvested from said culture, and whereby the present protein can be produced. The method for culturing the present transformant in a medium can be carried out according to a generally known method which is used for the culture of a host.

As a medium for culturing a transformant obtained by using a prokaryote such as *Escherichia coli* or the like or an eukaryote such as yeast or the like as a host, either a natural medium or a synthetic medium may be used as far as it contains a carbon source, a nitrogen source, an inorganic salt, etc. which are assimilable for said organism and in which the culturing of the transformant can be carried out efficiently.

As a carbon source, any carbon source will do as far as it is assimilable for said organism. For example, carbohydrates such as glucose, fructose, sucrose, molasses containing these, starch or starch hydrolysate, etc.; organic acids such as acetic acid, propionic acid, etc.; alcohols such as ethanol, propanol, etc.; etc. can be used.

As a nitrogen source, ammonium salts of inorganic acids or organic acids such as ammonia, ammonium chloride, ammonium sulfate, ammonium acetate, ammonium phosphate etc., other nitrogen-contained compounds, peptone, meat extract, yeast extract, corn steep liquor, casein hydrolysate, soybean meal or soybean hydrolysate, various microbial cells obtained by fermentation and their digests, etc. can be used.

As inorganic salts, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, magnesium phosphate, magnesium sulfate, sodium chloride, ferrous sulfate, manganese sulfate, copper sulfate, calcium carbonate, etc. can be used.

The culturing is carried out generally under aerobic conditions such as shake culture, deep aeration spinner culture or the like. The culture temperature is preferably 15 to 40°C, and the culturing time is generally 16 to 96 hours. During the culturing, the pH is kept at 3.0 to 9.0. The adjustment of the pH is carried out using an inorganic or an organic acid, an alkaline solution, urine, calcium carbonate, ammonia, etc.

Besides, if necessary, antibiotics such as ampicillin, tetracycline, etc. may be added to a medium during the culture.

In case of culturing a microorganism transformed with an expression vector using an inducible promoter, an inducer may be added to a medium, if necessary. For example, isopropyl- β -D-thiogalactopyranoside and the like in case of culturing a microorganism transformed with an expression vector using *lac* promoter and indole acrylic acid and the like in case of culturing a microorganism transformed with an expression vector using *trp* promoter may be added to the medium.

As a medium for culturing a transformant obtained using an animal cell as a host cell, a generally used RPMI1640 medium, an Eagle's MEM, a medium prepared by adding bovine fetal serum, etc. to these media or the like can be used.

The culturing is carried out usually under such conditions as in the presence of 5% CO₂ and the like. The culturing temperature is preferably 35 to 37°C, and the culturing time is generally 3 to 7 days.

Besides, if necessary, antibiotics such as kanamycin, penicillin, etc. may be added to a medium during the incubation.

As a medium for culturing a transformant obtained using an insect cell as a host, a generally used TNM-FH medium (manufactured by Pharmingen, Inc.), Sf-900 II SFM medium (manufactured by Life Technologies, Inc.), ExCell400 and ExCell405 (both manufactured by JRH Biosciences, Inc.), etc. can be used.

The culturing temperature is preferably 25 to 30°C, and the culturing time is generally 1 to 4 days.

Besides, if necessary, antibiotics such as gentamycin, etc. may be added to a medium during the culturing.

In order to isolate and purify a protein expressed according to the above method from the culture medium of the above transformant, generally known methods for enzyme isolation and purification may be used.

For example, in case that the present protein is expressed in cells in the soluble form, the cells are collected by centrifugation after the completion of culturing, suspended in an aqueous buffer, disrupted by an ultrasonic disrupter, a French press, a Manton Gaulin homogenizer, a Dymomill or the like to thereby obtain a cell-free extract solution. From the supernatant obtained by centrifuging said cell-free extract solution, a purified preparation can be obtained by employing generally known methods for enzyme isolation and purification, that is, methods such as solvent extraction, salting out with ammonium sulfate or the like, desalting, precipitation with an organic solvent, anionic exchange chromatography using resins such as diethylaminoethyl (DEAE)-Sephacrose, DIAION HPA-75 (manufactured by Mitsubishi Chemical Corporation) and the like, cationic exchange chromatography using resins such as S-Sephacrose FF (manufactured by Pharmacia) and the like, hydrophobic chromatography using resins such as butyl Sepharose, phenyl Sepharose and the like, gel filtration using molecular sieves, affinity chromatography, electrophoresis such as chromatofocusing, isoelectric focusing and the like independently or in combination.

Besides, in case that said protein is expressed intracellularly as an inclusion body, said protein is recovered according to a generally known method from precipitated fractions obtained similarly by disrupting cells after collection and then centrifuging the disrupted cells, and subsequently said protein as an inclusion body is solubilized with a protein denaturant. After structuring said protein to have a correct structure by diluting said solubilized solution to a protein denaturant-free solution or a solution in which the concentration of the protein denaturant is so lowered as not to denature the protein or by dialyzing said solubilized solution, a purified preparation can be obtained according to the same isolation and purification method described above.

In case that the present protein or its derivative such as a glycosylated form or the like is extracellularly secreted, said protein or its derivative such as a glycosylated form or the like can be recovered from the culture supernatant. That is, said culture is treated according to the same method described above such as centrifugation and the like to obtain soluble

fractions, from which a purified preparation can be obtained by employing the same isolation and purification method described above.

Besides, a protein expressed according to the above method can be produced also by chemical synthesis methods such as Fmoc method (fluorenylmethyloxycarbonyl method), tBoc method (t-butyloxycarbonyl method), etc. In addition, it is also possible to synthesize said protein by utilizing a peptide synthesizer available from Sowa Trading Co., Ltd. (manufactured by Advanced ChemTech, U.S.A.), Perkin-Elmer Japan (manufactured by Perkin-Elmer, U.S.A.), Pharmacia Biotech, K.K. (manufactured by Pharmacia Biotech, Sweden), Aloka Co., Ltd. (manufactured by Protein Technology Instrument, U.S.A.), Kurabo Industries Ltd. (Synthecell-Vega, U.S.A.), Nippon PerSeptive Ltd. (PerSeptive, U.S.A.), Shimadzu Corporation or the like.

3. Preparation of Alzheimer's disease related Antibody

As the antibody, any antibody such as a polyclonal antibody, a monoclonal antibody or the like will do as far as it can bind specifically to the above protein or polypeptide.

The polyclonal antibody can be prepared by separating and purifying sera obtained from an antigen-immunized animal. The monoclonal antibody can be prepared by fusing an antibody-producing cell obtained from an antigen-immunized animal with a myeloma cell to produce a hybridoma, culturing said hybridoma or inoculating the same to an animal to make the ascitic tumor and then separating and purifying said culture medium or said ascites.

The antigen can be prepared by purifying the protein of the present invention from various human cultured cells or by introducing a recombinant vector containing a DNA encoding a protein having the amino acid sequence represented by SEQ ID NO. 2 or a DNA encoding a protein having an amino acid sequence which includes substitution, deletion or addition of one or more amino acids of the amino acid sequence represented by SEQ ID NO. 2 and having apoptosis-suppressing activities into a host such as *Escherichia coli*, yeast, an animal cell, an insect cell or the like to make the host express the protein and then isolating and purifying the protein. In addition, the antigen can be prepared also by synthesizing a peptide having a partial sequence of the amino acid sequence represented by SEQ ID NO. 2 using an amino acid synthesizer.

As an immunization method, the antigen may be subcutaneously, intravenously or intraperitoneally administered to a non-human mammal such as a rabbit, a goat, a 3 to 20-week aged rat, mouse or hamster, or the like as it is. However, it is preferable that the antigen is administered either upon conjugating it to a highly antigenic carrier such as keyhole limpet hemocyanin, bovine serum albumin, bovine thyroglobulin or the like or together with an appropriate adjuvant such as Freund's complete adjuvant, aluminium hydroxide gel, pertussis vaccine or the like.

Administration of the antigen is carried out 3 to 10 times at 1 to 2 week intervals after the 1st administration. On the 3rd to 7th day after each administration, blood is collected from the venous plexus of ocular funds to examine whether or not said serum reacts with the

antigen used for immunization by measuring antibody titer according to an enzyme immunoassay (Antibodies—A Laboratory Manual, Cold Spring Harbor Laboratory, 1988) or the like. A non-human mammal whose serum shows an enough antibody titer against the antigen used for immunization is served as a supply source of the serum or the antibody-producing cell.

The polyclonal antibody can be prepared by separating and purifying said serum.

The monoclonal antibody can be prepared by fusing an antibody-producing cell obtained from an antigen-immunized animal with a myeloma cell to produce a hybridoma, culturing said hybridoma or inoculating the same to an animal to make an ascitic tumor and then separating and purifying said culture medium or said ascites.

As the antibody-producing cell, a splenocyte cell, an antibody-producing cell in a lymph node or the peripheral blood, particularly a splenocyte is used suitably.

As the myeloma cell, a mouse-derived established cell line such as P3-X63Ag8-U1(P3-U1) [*Current Topics in Microbiology and Immunology*, 18, 1 - 7 (1978)], P3-NS1/1-Ag41(NS-1) strain [*European J. Immunology*, 6, 511 - 519 (1976)], SP2/0-Ag14(SP-2) strain [*Nature*, 276, 269 - 270 (1978)], P3-X63-Ag8653(653) [*J. Immunology*, 123, 1548 - 1550 (1979)], P3-X63-Ag8(X63) [*Nature*, 256, 495 - 497 (1975)] or the like as an 8-azaguanine-resistant mouse (originated from BALB/c) myeloma cell line is used suitably.

The hybridoma cell can be made according to the following procedures.

An antibody-producing cell and a myeloma cell are mixed, suspended in a HAT medium (a medium prepared by adding hypoxanthine, thymidine and aminopterin to a normal medium) and then cultured for 7 to 14 days. After culturing, a part of the culture supernatant is subjected to an enzyme immunoassay or the like to select a cell which is reactive to an antigen but unreactive to an antigen-free protein. Subsequently, the selected cell is cloned by limiting dilution, followed by enzyme immunoassay to select a clone which shows a stably high antibody titer as a monoclonal antibody-producing hybridoma cell.

The monoclonal antibody can be prepared by separating and purifying a culture medium obtained by culturing the hybridoma or ascites obtained by intraperitoneally inoculating the hybridoma cell to an animal to make the animal ascitic cancer.

As a method for separating and purifying a polyclonal antibody or a monoclonal antibody, treatment by employing methods such as centrifugation, ammonium sulfate precipitation, caprylic acid precipitation, chromatography using a DEAE-Sepharose column, an anion exchange column, a protein A or G column, a gel filtration column, etc., independently or in combination can be given.

4. Utilization of Human Nap1 DNA, Protein or Antibody

(1) The mRNA of human Nap1 gene of the present invention can be detected by using the DNA described in 1 above according to the Northern hybridization method (Molecular Cloning, Second Edition), the PCR method [PCR Protocols (1990)], the RT

(reverse-transcribed) -PCR method, etc.

(2) Brain tissues of healthy people and those of the subjects are screened for the human Nap1 gene mRNA according to the method described in (1) above and then the healthy people and the subjects are compared for the mRNA abundance to examine whether or not the abundance is decreased in the subjects, and whereby the diagnosis of Alzheimer's disease in the subjects can be performed.

In the case of Northern hybridization method, the abundance of mRNA can be determined by measuring the quantity of the hybridized probe in dependence on the label of said probe, for example, the radioactivity in the case of ^{32}P label and the intensity of fluorescence in the case of a fluorescent label. In the case of RT-PCR method, it can be determined, for example, by staining an amplified fragment with a DNA-specific fluorescent stain such as ethidium bromide, SYBR Green I or the like and subsequently measuring the intensity of fluorescence.

(3) Using the DNA of the present invention, the chromosomal location of human Nap1 gene can be determined according to somatic cell hybrid method [*Annual Review of Genetics*, 9, 407 (1975)] or *in situ* hybridization method.

In the somatic cell hybrid method, the number of a chromosome where the human Nap1 gene exists is determined as follows. Firstly, the subculture of a hybrid cell, in which a human cell and a mouse or hamster established cell line are fused, brings a hybrid cell clone in which only 1 or 2 human chromosomes remain, the remaining chromosome(s) is (are) specified to prepare such a panel of a series of hybrid cell clones which covers 23 human chromosomes. Such a hybrid cell panel is commercially available from Bios Laboratories, Inc. or the like. A genome DNA is prepared from each cell in the panel, followed by carrying out PCR serving this genome DNA as a template and using a human Nap1-specific primer to thereby examine the amplification of a DNA fragment corresponding to the human Nap1 gene. Then, it can be judged that the human Nap1 gene located on the human chromosome contained in a cell where amplification took place.

On the other hand, in the *in situ* hybridization method [*Annals of Human Genetics*, 45, 135 (1981); *Cell*, 52, 51 (1988)], first of all, the hybridization is carried out to a human chromosome specimen using the human Nap1 DNA of the present invention as a probe. Then, a signal of hybridization to the probe is detected to specify the location of the signal on the specimen, and whereby not only the chromosome where the human Nap1 gene located but also the physical location on said chromosome can be specified. The probe is labeled with a radioactive isotope ^3H or biotin, and a signal can be detected by autoradiography in the case of ^3H labeling and by avidin labeled with a fluorescent pigment FITC in the case of biotin labeling.

(4) The antisense oligonucleotide described in 1 above is introduced into a cell to repress the transcription or the translation of the Nap1 gene, and whereby apoptosis can be induced in the cell. The introduction of the antisense oligonucleotide can be carried out in the same manner as that of a recombinant vector. Besides, as methods for detecting the

apoptosis of a cell, (i) microscopic observation, (ii) TUNEL method, (iii) DNA ladder detection method, (iv) quantitation of DNA fragmentation ratio, (v) cell size distribution measurement, etc. can be enumerated [Latest Apoptosis Experimentation Techniques, Youdo-sha (1995)].

In microscopic observation, morphological cell changes characteristic for apoptosis, for example, condensation of a nucleus, condensation of a chromatin, atrophy of an organelle, formation of an apoptotic body, shrinkage of the whole cell, etc. are observed. The TUNEL method is a method for detecting the digested end of a DNA to be formed by apoptosis by labeling said end with biotin or digoxigenin using terminal deoxynucleotidyl transferase (TdT). The DNA ladder detection method is a method for detecting the fragmentation of a chromatin DNA resulted from apoptosis by extracting a DNA from a cell and electrophoresing the extracted DNA on an agarose gel, and whereby the fragmented DNA is detected in a ladder state. The quantitation of DNA fragmentation ratio is a method by which only a fragmented, low molecular weight DNA is extracted and said DNA is quantitated using diphenylamine. The cell size distribution measurement is a method by which the increase of small size cells caused by contraction or fragmentation of cells resulting from apoptosis is measured utilizing a particle size analyzer such as Coulter multisizer or the like. According to these methods, whether or not apoptosis takes place in a cell can be detected.

(5) By repressing the transcription of DNA or the translation of mRNA using the antisense oligonucleotide (RNA/DNA) described in 1 above [*Kagaku* (Chemistry), 46, 681 (1991); *Bio/Technology*, 9, 358 (1992)], it is possible to induce apoptosis and to utilize the same for the treatment of diseases caused by the participation of apoptosis suppression in the state of diseases.

For example, the oligonucleotide is injectionally administered to a patient in the affected part or systemically together with an appropriate carrier having a high affinity for cell membranes such as liposome, cholesterol or the like in order to facilitate its incorporation into cells and is made to be incorporated into the patient's cells to induce apoptosis, and whereby diseases can be treated.

As examples of the diseases the state of which the suppression of apoptosis participates in, cancers, autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis and the like, etc. can be given.

In many cancer cells, mutations such as deletion and the like in genes such as p53 which is an apoptosis-inducing factor have been found [*British Journal of Cancer*, 68, 653 (1993)]. And, it is considered that, in such cells, apoptosis mechanism which, in normal tissues, excludes cells having abnormalities such as gene mutation and the like becomes unworkable to thereby advance the proliferation of cancer cells. In addition, there also is a report that the antisense oligo DNA of bc1-2 which works as an apoptosis-suppressing gene in many cells can inhibit the growth of human colon cancer in an animal experiment (NIKKEI'S DICTIONARY OF LATEST BIO TERMINOLOGY, Nikkei BP Inc., 1995).

Therefore, the antisense oligonucleotide of the present invention can be utilized for the treatment of apoptosis-related cancers by suppressing the expression of the gene of the present invention using said antisense oligonucleotide to induce apoptosis.

On the other hand, it is considered that autoimmune diseases outbreak because autoreactive T cells, which are generally excluded by apoptosis, are not excluded, and it has been known that a mouse possessing a mutated Fas gene, which is regarded as an important apoptosis-inducing gene in this mechanism, or a mutant of a gene encoding a ligand of the Fas protein shows symptoms of autoimmune diseases because the expression of the Fas gene or Fas protein is repressed [*Nature*, 356, 317 (1992) and *Cell*, 75, 1169 (1993)].

Therefore, autoimmune diseases can be treated by suppressing the expression of the gene of the present invention using the antisense oligonucleotide of the present invention to thereby induce apoptosis.

(6) Human Nap1 protein can be obtained using the DNA described in 1 according to the method described in 2.

(7) The protein described in 2 can be utilized for the treatment of Alzheimer's disease by administering the same to the brain of an Alzheimer's disease patient to suppress the apoptosis of neuronal cells. In addition, the suppression of cell apoptosis can be expected by the administration of this protein, so that it can be utilized for the treatment of diseases the state of which the induction of apoptosis participates in.

A therapeutic composition for apoptosis-participating diseases which contains the protein of the present invention as an active ingredient can generally be systemically or focally administered parenterally. As a parenteral administration method, intravenous injection such as drip infusion or the like, intramuscular injection, intraperitoneal injection or subcutaneous injection can be selected, and it can be selected suitably in response to ages and symptoms of patients. The dosage of said composition differs in age, administration route and administration time and thus can be changed widely. In this case, the effective dose of the protein of the present invention and the effective dose to be administered as a composition with an appropriate diluent and a pharmacologically adoptable carrier are selected from the range of 10 μ g to 10mg/kg weight/time.

As a dosage form to be applied to the case of parenterally administering the protein of the present invention, generally a unit-dose ampoule or a multiple-dose container (a vial) which each contains additives such as a stabilizer, a buffer, a preservative, a tonicity agent, etc. is given.

Furthermore, a therapeutic composition containing the protein of the present invention for apoptosis participating diseases may contain a pharmaceutically acceptable carrier and additives according to its administration route. As examples of such a carrier and additives, water, pharmaceutically acceptable organic solvents, collagen, polyvinyl alcohol, polyvinylpyrrolidone, sodium carboxymethylcellulose, sodium polyacrylate, sodium alginate, water soluble dextran, sodium carboxymethyl starch, pectin, methylcellulose, ethylcellulose, xanthane gum, gum arabic, casein, gelatin, agar, glycerin, polyethylene glycol, vaseline,

paraffin, stearic acid, human serum albumin (HSA), mannitol, sorbitol, lactose, surfactants acceptable as pharmaceutical additives, etc. can be given. An additive to be used, though it is selected from the above suitably or in combination according to the dosage form of the present invention, is nowise restricted to these.

As diseases to which the administration of the protein of the present invention is applied, that is, diseases the state of which the induction of apoptosis participates in, progressive neuro degenerative diseases, e.g., Parkinson's disease [*Journal of Neurological Sciences*, 137, 120 (1996)] and amyotrophic lateral sclerosis [*Neuropathology and Applied Neurobiology*, 21, 498 (1995)]; Huntington's chorea [*Experimental Neurology*, 133, 265 (1995); *Neuroreport*, 6, 1053 (1995) and *Journal of Neuroscience*, 15, 3775 (1995)]; retinitis pigmentosa [*Neuron*, 11, 595 (1993) and *Proc. Natl. Acad. Sci. USA*, 91, 974 (1994)]; glaucoma [*Investigative Ophthalmology and Visual Science*, 36, 774 (1995) and *Journal of Glaucoma*, 5, 345 (1996)]; alcoholic hepatitis [*Journal of Hepatology*, 6, 137 (1988) and *Journal of Pathology*, 171, 73 (1993)]; etc. are given.

(8) An antibody can be produced using the protein described in 2 as an antigen according to the method described in 3.

(9) Using the antibody described in 3, the human Nap1 protein can be detected.

Specifically, detection methods employing ELISA method and fluorescent antibody method using a microtiter, Western blot method, etc. can be enumerated.

(10) The antibody described in 3 can be utilized for immunological tissue staining of the brain tissues of subjects.

(11) Alzheimer's disease in a subject can be diagnosed by detecting human Nap1 protein derived from the brain tissue of a healthy person and that of a subject, measuring the quantities of said protein in the both brains and comparing the quantities to examine the decrease of said quantity in the subject.

(12) The antibody described in 3 can be utilized for the treatment of diseases the state of which the suppression of apoptosis participates in because it is expected to suppress the human Nap1 activity of cells to induce apoptosis by the administration of the antibody described in 3.

The dose and the dosage form are the same as aforementioned.

As examples of apoptosis-participating diseases, cancers; autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, etc.; etc. can be given.

[Examples]

Hereinafter, the present invention will be described further specifically by means of examples. However, the technical scope of the present invention is nowise limited to these examples.

Example 1 Cloning of cDNA Fragment of Human Nap1 Gene

(1) Preparation of the total RNA from the brain

From the cerebral cortex of 10 sporadic Alzheimer's disease patients and 3 non-Alzheimer's disease subject, the total RNAs were obtained using TRIzol reagent (manufactured by Life Technologies). Specific methods employed were in accordance with the instructions given in the manual of the reagent. These RNAs were treated with ribonuclease-free deoxyribonuclease (manufactured by Promega) to remove contaminating DNAs, extracted with phenol-chloroform, precipitated with ethanol and then dissolved in distilled water treated with diethyl pyrocarbonate (DEPC).

(2) Differential display using the total RNA

According to the method of Ito et al. [*FEBS Letters*, 351, 231 (1994)], differential display was carried out. That is, 2.5 μ g each of the RNAs obtained in (1) were mixed with 50pmol of one anchor primer out of three primers of SEQ ID NOS. 4 to 6, followed by addition of DEPC-treated distilled water to give a total volume of 10 μ l. After heating at 70 °C for 10 minutes, each RNA solution was chilled by immediately immersing in ice water. In this chilled solution, 10 μ l of 2 \times reverse transcriptase reaction buffer [40mM Tris-HCl (pH 8.4), 100mM KCl, 5mM MgCl₂, 2 μ l of 20mM dithiothreitol (DTT), 0.2mg/ml bovine serum albumin (BSA), 1mM dNTP (dATP, dGTP, dCTP, dTTP)] containing 200 units of SUPERScript II RNase HReverse Transcriptase (manufactured by Life Technologies) as a reverse transcriptase was mixed, reacted at 25°C for 10 minutes and at 42°C for 50 minutes to synthesize a cDNA, heated at 90°C for 5 minutes and then stopped the reaction. To this reaction solution, 80 μ l of TE buffer [10mM Tris-HCl (pH 8.0), 1mM EDTA] was added to dilute 5-fold.

To 2 μ l each of the synthesized cDNA, 2 μ l of 10 \times PCR buffer [100mM Tris-HCl (pH 8.8), 500mM KCl, 15mM MgCl₂, 1% Triton X-100, 1mM dNTP], 5pmol of an anchor primer (the same primer used in cDNA synthesis), 10pmol of an arbitrary primer (one out of 10 primers of SEQ ID NOS. 7 to 16), 1 unit of Gene Taq DNA polymerase (manufactured by Nippon Gene) were added and followed by addition of distilled water to give a total volume of 20 μ l, which was then placed in a GeneAmp PCR System 9600 PCR apparatus (manufactured by Perkin Elmer).

PCR was carried out on condition that, after the reactions at 94°C, 37°C and 72°C for each 2 minutes, a cycle of reaction steps consisting of 94°C for 30 seconds, 55°C for 1 minute and 72°C for 1 minute was repeated 25 times and followed by the final reaction at 72 °C for 5 minutes. Since 3 kinds of anchor primers and 10 kinds of arbitrary primers were used, 30 sets of samples were subjected to the PCR.

To 2 μ l each of reaction solutions after PCR, 2 μ l of formamide dye solution (98% formamide, 0.01% methyl violet, 10mM EDTA) was added. The mixture was heated at 90 °C for 2 minutes and then ice-cooled. The reaction solution was electrophoresed on a 6% acrylamide gel (20cm wide \times 33cm high \times 0.35mm thick) at 20mA for 2 hours using a Tris-Glycine buffer. After fluorescently staining DNA with 0.01% SYBR Green I (manufactured by Molecular Probe), approx. 1,200 fragments amplified by PCR were

detected and compared using Fluorimager 575 (manufactured by Molecular Dynamics). Out of the above fragments, a band showing remarkable reduction common throughout 10 cases of the brains of sporadic Alzheimer's disease patients in comparison with 3 cases of the brains of non-Alzheimer's disease subjects was selected. The amplification product which contained this band was obtained by the PCR using the primer of SEQ ID NO. 4 as an anchor primer and that of SEQ ID NO. 7 as an arbitrary primer.

(3) Cloning of selected amplified fragment band

The picture of the whole gel containing the selected band was printed out to have a actual-size enlargement, which was accurately superposed upon the gel to cut out the selected band from the gel. The remaining gel was scanned by a fluorimager to confirm that the band was cut out. After adding the gel cut out to 50 μ l of a PCR solution [1 \times PCR buffer containing an anchor primer (SEQ ID NO. 4) and an arbitrary primer (SEQ ID NO. 7)], 1 unit of a Gene Taq DNA polymerase was added thereto to carry out PCR, and whereby fragments contained in the gel was further amplified. PCR was carried out on condition that a cycle of reaction steps consisting of 94°C for 30 seconds, 55°C for 1 minute and 72°C for 1 minute was repeated 30 times. The solution after reaction was precipitated with ethanol and then dissolved in 10 μ l of TE buffer.

One μ l of the amplified fragment and 1 μ l of a PCR fragment cloning vector pT7Blue T-Vector (manufacture by Novagen; a vector prepared by digesting the *Eco*R V site of a plasmid T7Blue and adding one dT to the 3'-end of the both strand) were mixed, followed by ligating the amplified fragment into the plasmid using a DNA ligation kit Ver. 2 (manufactured by Takara Shuzo K.K.) according to the instructions given in the kit. *Escherichia coli* DH5 α (manufactured by Life Technologies) was transformed to obtain an ampicillin-resistant strain. Said transformant colony was pricked with a sterilized tooth pick and suspended in 25 μ l of PCR solution, followed by addition of 1 unit of Gene Taq DNA polymerase to carry out PCR under the same conditions as the reamplification of the amplified fragment. Two μ l of this solution and 2 μ l of PCR solution used when this amplified fragment was obtained in (2) were juxtaposed on the same gel to carry out the same electrophoresis and fluorescent staining as the differential display, followed by detecting an amplified fragment by a fluorimager. Because the fragment was amplified on quite the same position as the selected band observed in the PCR solution described in (2) according to PCR using a colony as a template, it was judged that said transformant contained the DNA of the selected band.

This transformant was named Clone 9-1, from which plasmid p9-1 was isolated.

(4) Determination of the nucleotide sequence of amplified fragment

The nucleotide sequence of the amplified fragment contained in p9-1 was determined. The reaction for sequencing was carried out using SequiTherm Long-Read Cycle Sequencing Kit (manufactured by Epicentre Technologies), and the sequence was determined using ALF

DNA Sequencer (manufactured by Pharmacia). The reagents and the methods employed were in accordance with the instructions given in the kit. The obtained nucleotide sequence was represented by SEQ ID NO. 3. Said nucleotide sequence comprised 265 nucleotides, which corresponded to the 3986th to the 4236th of the nucleotide sequences represented by SEQ ID NO. 1. Incidentally, the 1st to the 15th of the sequence represented by SEQ ID NO. 3 are derived from the arbitrary primer (SEQ ID NO. 7) and the 241st to the 265th of the sequence are derived from the anchor primer (SEQ ID NO. 4), so that the 1st to the 7th and the 259th to the 265th of the sequence represented by SEQ ID NO. 3 do not correspond to the base sequence represented by SEQ ID NO. 1.

Example 2 Confirmation of Expression Specificity of Clone 9-1 Gene by RT-PCR

In addition to the total RNAs from the cerebral cortices of 10 sporadic Alzheimer's disease patients and 3 non-Alzheimer's disease subjects isolated in Example 1, the total RNAs were isolated from the cerebral cortices of 7 more non-Alzheimer's disease subjects in the same manner as in (1) of Example 1. From 1 μ l each of these total RNAs, a single-stranded cDNA was synthesized using SUPERSCRIPT II RNase H reverse transcriptase and a random hexamer primer (manufactured by Life Technologies). The specific reagents and methods employed were in accordance with the instructions given in the manual attached by Life Technologies.

The Alzheimer's disease patients were compared with the non-Alzheimer's disease subjects with respect to the abundance of mRNA of Clone 9-1 gene in the cerebral cortex. That is, using 2 μ l of the solution after reaction, PCR was carried out in 1 \times PCR buffer by adding thereto 10pmol of 5'-end sense primer (SEQ ID NO. 17), 10pmol of 3'-end antisense primer (SEQ ID NO. 18) and 1 unit of Gene Taq DNA polymerase. PCR was carried out on condition that, after the heating at 94°C for 3 minutes, a cycle of reaction steps consisting of 94°C for 30 seconds, 55°C for 1 minute and 72°C for 1 minute was repeated 28 times and followed by the final reaction at 72°C for 5 minutes.

After electrophoresing on a 1% agarose gel, the gel was stained with 0.01% SYBR Green I to detect bands for the amplified fragment (corresponding to the 27th to the 211th of the nucleotide sequence represented by SEQ ID NO. 3 and the 4005th to the 4189th of the nucleotide sequence represented by SEQ ID NO. 1).

As a consequence, the results shown in Fig. 1 were obtained.

Fig. 1 is electrophoretograms showing the results of RT-PCR using cerebral cortex RNAs of Alzheimer's disease patients and non-Alzheimer's disease subjects with respect to Clone 9-1 cDNA (=human Nap-1 gene cDNA), wherein AD 1 to 10 of the lane indicates the cerebral cortex RNAs of Alzheimer's disease patients and normal 1 to 10 indicates those of non-Alzheimer's disease subjects, and 9-1 indicates the results in the case of having used a Nap-1 gene-specific primer to the foregoing cerebral cortex RNAs and ef1 α indicates the results in the case of having used a primer specific to an elongation factor 1 α gene used as a control to said RNAs.

From the results shown in Fig. 1, the quantity of amplified fragment is decreased in all of 10 sporadic Alzheimer's disease patients as compared with 10 non-Alzheimer's disease subjects as controls, from which it is confirmed that the abundance of mRNA of the Clone 9-1 gene is decreased in the cerebral cortex of Alzheimer's disease patients.

Incidentally, the unchangingness of abundance of mRNA itself was confirmed by carrying out the same RT-PCR with respect to an elongation factor 1 α (ef1 α) gene which is a housekeeping gene using ef1 α -specific primers represented by SEQ ID NOS. 19 and 20. Said PCR was carried out on condition that, after the heating at 94°C for 3 minutes, a cycle of reaction steps consisting of 94°C for 30 seconds, 58°C for 1 minute and 72°C for 2 minutes was repeated 25 times and followed by the final reaction at 72°C for 5 minutes.

Example 3 Cloning of Full-length cDNA of Clone 9-1

(1) Isolation of cDNA clones from cDNA library

Clones of cDNA was isolated from a human cerebral cortex cDNA library (manufactured by CLONETECH) by plaque hybridization. A nylon membrane filter (Hybond N+; manufactured by Amersham) on which 2×10^5 plaques were blotted was sealed in a hybridization solution [$6 \times$ SSC, $10 \times$ Denhart's solution (0.2% BSA, 0.2% Ficoll, 0.2% polyvinyl pyrrolidone), 1% (w/v) SDS, 200 μ g/ml of salmon sperm DNA denatured (heated for 5 minutes in boiled water and the rapidly cooled in ice) after ultrasonic treatment] and prehybridized at 60°C for 2 hours.

Probes were made as follows. That is, PCR was carried out in $1 \times$ PCR buffer in the same manner as in Example 1 using the primers of SEQ ID NOS. 21 and 18 and serving the plasmid DNA of Clone 9-1 as a template, thereby amplifying the cDNA moiety corresponding to the 26th to the 211th of the nucleotide sequence represented by SEQ ID NO. 3. PCR was carried out on condition that a cycle of reaction steps consisting of 94°C for 20 seconds, 58°C for 30 seconds and 72°C for 1 minute was repeated 30 times.

The solution after PCR was electrophoresed on a 6% acrylamide gel, the band for the amplified fragment was cut out from the gel, and PCR was repeated under the same conditions serving this cut gel as a template to reamplify the fragment. The reaction solution was precipitated with ethanol, followed by dissolving in 10 μ l of distilled water. To 1 μ l of this solution, 5 μ l of $10 \times$ buffer [100mM Tris-HCl (pH 8.8), 500mM KCl, 1% Triton X-100], each 1 μ l of 10pmol primers (SEQ ID NOS. 22 and 23), 1 μ l of 4mM (dATP, dGTP, dTTP), 10 μ l of dCTP (manufactured by NEN) and 1 μ l of Gene Taq were added to carry out PCR again under the same conditions, thereby making a 32 P-labeled probe.

From the reaction solution, a probe was purified using a quick spin column G-50 (DNA) (manufactured by Boehringer Mannheim). This probe was added to the hybridization solution, and the filter was sealed therein and hybridized overnight at 60°C. After washing the filter with $0.2 \times$ SSC containing 0.2% (w/v) SDS at 60°C for 30 minutes, an imaging plate (manufactured by Fuji Photo Film Co., Ltd.) was exposed and positive plaques were detected using Bioimaging Analyzer BAS2000A (manufactured by Fuji Photo

Film Co., Ltd.) and then isolated. Since this cDNA library uses λ ZAP II as a vector, *in vivo* excision was carried out using the isolated positive plaques, and whereby an *Escherichia coli* transformant (*Escherichia coli* SOLR/p9-1-6) containing a cDNA clone p9-1-6 whose vector moiety was changed from λ ZAP II to plasmid pBluescript II was obtained. Specific methods for the *in vivo* excision employed were in accordance with those given in the manual of Stratagene.

Incidentally, the *Escherichia coli* transformant *Escherichia coli* SOLR/p9-1-6 was internationally deposited as FERM BP-6201 in National Institute of Bioscience & Human-technology, Agency of Industrial Science & Technology (Higashi 1-1-3, Tsukuba City, Ibaraki, Japan) on December 10, 1997.

(2) Analysis of nucleotide sequence of cDNA contained in p9-1-6

The nucleotide sequence of the p9-1-6 cDNA was determined in the same manner as in (4) of Example 1. As a result of it, a 4071-bp nucleotide sequence corresponding to the 166th to the 4236th of SEQ ID NO. 1 could be determined, but the nucleotide sequence from here to the 5'-end could not be determined.

(3) Isolation of cDNA clone by 5'-RACE

In order to clarify the nucleotide sequence of the whole cDNA, 5'-RACE was carried out. With respect to human brain poly(A)⁺ RNA (manufactured by Clontech), approx. 0.3-kb DNA fragment considered to contain cDNA on the 5'-end side from the cDNA moiety corresponding to the primer sequence (corresponding to the 314th to the 338th of the nucleotide sequence represented by SEQ ID NO. 1) was amplified by using the cDNA-specific primer described in SEQ ID NO. 22 and 5'-RACE System as a 5'-RACE kit manufactured by Life Technologies.

Specific methods of the 5'-RACE employed were in accordance with the instructions given in the kit's manual. This DNA fragment was cloned into a vector pT7Blue T-Vector in the same manner as in (3) of Example 1 to obtain pRCOM.

A pRCOM-containing *Escherichia coli* transformant *Escherichia coli* DH5 α /pRCOM was internationally deposited as FERM BP-6202 in National Institute of Bioscience & Human-technology, Agency of Industrial Science & Technology (Higashi 1-1-3, Tsukuba City, Ibaraki, Japan) on December 10, 1997.

(4) Sequencing of cDNA

The nucleotide sequence of cDNA of the clone pRCOM obtained by 5'-RACE was determined in the same manner as described in (5) of Example 1, and this nucleotide sequence and the nucleotide sequence of the p9-1-6 cDNA determined in (2) of Example 4 were ligated to obtain a nucleotide sequence considered to be the full-length cDNA of 9-1. This nucleotide sequence consists of 4236 base pairs, in which an open reading frame (ORF) consisting of 1128 amino acids existed. This amino acid sequence was represented by SEQ

ID NO. 2.

As a result of comparing this nucleotide sequence with the nucleotide sequence data base GenBank using a homology searching program BLAST, a nucleotide sequence identical with said nucleotide sequence was absent in the known nucleotide sequences except some, for example, those of Access Nos. AA307472, R58000, N44074, etc. were found in EST, and thus this nucleotide sequence was a novel one. Incidentally, ESTs which have identical sequence with 9 – 1 were considered to be of nonfull-length cDNAs because the identical sequence occurred from the midway of the ORF. In addition, it was rat Nap1 gene that showed the highest homology among the known nucleotide sequences.

The results were shown in Fig. 2. Fig. 2 is a list showing the comparison of the amino acid sequences of human Nap1 and rat Nap1. By single letter notation, the amino acid sequence of the human Nap1 was shown in the row marked as 'human' and that of the rat Nap1 in the row marked as 'rat', provided that, in the case of the rat Nap1, the same amino acids as in the case of the human Nap1 were marked by asterisk and different amino acids alone were noted by single letters. The numbers given on the right ends show the number of amino acids.

As shown in Fig. 2, the amino acid sequence of ORF has 99.2% homology with that of rat Nap1 ORF, and the number of its amino acids was also 1128 which was quite the same. Therefore, this cDNA was considered to be the cDNA of the human Nap1 gene.

Example 4 Determination of Chromosomal location of Human Nap1 Gene

The chromosomal location of the human Nap1 gene was determined according to the method of Ruddle et al. [*Annual Review of Genetics*, 9, 407 (1975)] by using a human-rodent hybrid cell panel (manufactured by BIOS Laboratories).

That is, genome DNAs were purified from hybrid cells respectively corresponding to human chromosomes. To 100ng each of said chromosomal DNAs, PCR was carried out using a sense primer (SEQ ID NO. 23) and an antisense primer (SEQ ID NO. 18) amplifying the 3'-end region, which was not preserved between human and rat Nap1 genes (corresponding to the 3837th to the 4189th of the nucleotide sequence represented by SEQ ID NO. 1) out of the nucleotide sequence of the human Nap1 gene, and an EX Taq DNA polymerase (Manufactured by Takara Shuzo K.K.).

PCR was carried out on condition that the reaction was firstly conducted at 94°C for 1 minute, then a cycle of reaction steps consisting of 94°C for 1 minute, 61°C for 1 minute and 72°C for 1.5 minutes was repeated 30 times and followed by the reaction at 72°C for 5 minutes. As a result, amplification was observed only in a DNA containing human chromosome 2, and thus the human Nap1 gene was considered to exist in the chromosome 2.

In order to determine the chromosomal location of the Nap1 gene in further detail, fluorescence *in situ* hybridization (FISH) was carried out based on literature [*Genomics*, 17, 153 (1993)]. That is, a human metaphase chromosome was prepared from lymphocytes of a normal male according to thymidine synchronization, and an R band stained specimen was

made according to bromodeoxyuridine releasing method. Before hybridization, the chromosome was stained with Hoechst 33258 and then irradiated with UV light. Approximately 4.2kb of hNAP1 cDNA was labeled with biotin according to nick translation using biotin-16-dUTP for a probe. The biotin-labeled probe was added to a hybridization solution [50% formamide, 10% dextran sulfate (manufactured by Sigma), $2\times$ SSC, $2\mu\text{g}/\mu\text{l}$ of salmon sperm DNA denatured after sonication, $2\mu\text{g}/\mu\text{l}$ of *Escherichia coli* tRNA] to give a final concentration of $25\text{ ng}/\mu\text{l}$, thereby hybridizing to the denatured chromosome specimen.

According to the method described in the literature [Proc. Natl. Acad. Sci. USA, 83 2934 (1986)], the hybrid was reacted with FITC-labeled avidin (manufactured by Boehringer Mannheim) and further with biotin-labeled anti-avidin D (manufactured by Vector) in order to amplify the fluorescence, followed by reacting with FITC-labeled avidin again to detect the hybridized site. The chromosome was stained with $1\mu\text{g}/\mu\text{l}$ propidium iodide.

As a result, it was found that the chromosomal position of the present gene was q-32 of the long arm of the 2nd chromosome and that it existed in 2q32.1 - 2q32.2.

Example 5 Detection of Expression of Human Nap1 mRNA in Various Tissues

(1) Northern blotting of human Nap1 gene in various human tissues

In order to examine the expression of Nap1 gene in various human tissues, the expression of Nap1 mRNA in various human tissues was detected by Northern blotting using Clontech's Filter MTN (Multiple Tissue Northern) Blots on which mRNAs of various human tissues are blotted. In the Human MTN Blot used, $2\mu\text{g}$ each of mRNAs of heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas; in the Human NTN Blot II used, $2\mu\text{g}$ each of mRNAs of spleen, thymus, prostate gland, testis, ovary, small intestine, large intestine and peripheral blood leukocyte; and in the Human Brain MTN Blot III, $2\mu\text{g}$ each of mRNAs of cerebellar tonsil, caudate nucleus, corpus callosum, hippocampus, hypothalamus (the whole brain), substantia nigra, subthalamus (nucleus subthalamicus) and thalamus are blotted.

In order to use the Clone 9-1 cDNA fragment obtained in Example 1 as a probe, said cDNA fragment was labeled with ^{32}P using [α - ^{32}P]dCTP and a Prime-It II labeling kit (manufactured by Stratagene). The specific reagents and methods for labeling employed were in accordance with the instructions given in the kit.

A filter was immersed and sealed in a hybridization solution [$6\times$ SSC, $10\times$ Denhart's solution, 1% SDS, $200\mu\text{g}/\text{ml}$ of salmon sperm DNA denatured (heated for 5 minutes in boiled water and the rapidly cooled in ice) after ultrasonic treatment] and prehybridized at 60°C for 2 hours. Next, the probe was heated for 5 minutes in boiled water, denatured by rapidly cooling in ice and added to the hybridization solution, and the filter was immersed and sealed in the solution and hybridized overnight at 60°C . The filter was taken out from the solution and washed by shaking in $0.2\times$ SSC containing 0.2% SDS at 60°C for 30 minutes. The filter was superposed upon a imaging plate (manufactured by Fuji Photo Film Co., Ltd.)

to carry out autoradiography, followed by analysis using Bioimaging Analyzer BAS2000A (manufactured by Fuji Photo Film Co., Ltd.).

The results are shown in Fig. 3. In Fig. 3, the respective bands show, from the left, the results of Northern Blotting in cerebellar tonsil, caudate nucleus, corpus callosum, hippocampus, hypothalamus (the whole brain), substantia nigra, subthalamus (nucleus subthalamicus), thalamus, spleen, thymus, prostate gland, testis, ovary, small intestine, large intestine, peripheral blood leukocyte, heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. The arrow indicates the position of human Nap 1 mRNA. The bands for *efl* α shown beneath the respective bands are the results of Northern blotting of the elongation factor 1 α gene as a control.

As shown in Fig. 3, bands corresponding to 4.2-kb mRNA were detected in all the tissues excluding the peripheral blood leukocyte as to the human Nap1 gene. Therefore, it was found that the human Nap1 gene was a gene which is expressed ubiquitously in each tissue. Among the tissues, heart and skeletal muscle exhibited high expression. In addition, in respective tissues of the brain, it was expressed ubiquitously in all of the tissues.

(2) RT-PCR of Nap1 gene in human neuronal cell line

In which of a neuronal cell and a glial cell expression will be observed mainly in brain was examined by RT-PCR of cell lines. SH-SY5Y [donated by Dr. Shinichi KOUSAKA of National Institute of Neurological Science; *Cancer Research*, 33, 2643 (1973)] and LA-N-5 [donated by Dr. Takeshi YAMADA of Kyushu University; *Experimental Cell Research*, 148, 21 (1983)] as neuroblastoma cell lines and U-251 [donated by Dr. Takeshi YAMADA of Kyushu University; *Journal of Neuropathology & Experimental Neurology*, 40, 390 (1981)] as a glioma cell line were respectively cultured in DMEMs (Dulbecco's modified Eagle's medium) each containing 10% bovine fetal serum, 100 units/ml penicillin and 100 mg/ml Streptomycin at 37°C in a 5% CO₂ incubator. The total RNAs were isolated from the respective cells and subjected to RT-PCR in the same manner as in Example 2.

The results are shown in Fig. 4. In Fig. 4, SH-SY5Y, LA-N-5 and U-251 described on the left indicate the aforementioned respective cells, hNAP-1 indicates the results of having used a human Nap1 gene-specific primer and *efl* α indicates the results of having used a primer specific to the elongation factor 1 α gene used as a control.

As shown in Fig. 4, the expression of the Nap1 gene was detected in SH-SY5Y and LA-N-5 as neuroblastoma cell lines but not detected in U-251 as a glioma cell lines. Therefore, it was considered that the gene of the present invention was expressed mainly in a neuronal cell rather than in a glial cell.

(3) *In situ* hybridization of human Nap1 gene in brain

In order to confirm that the gene of the present invention is expressed in the brain (mainly in nerve cells), *in situ* hybridization was carried out in human cerebral tissues.

Human cerebral tissues to be served as specimens are tissues of spinal cord, cerebellum,

mesencephalon, basal ganglia, frontal cerebral cortex region and hippocampus, which were used by fixing with formalin after death and then embedding in paraffin.

First of all, PCR was carried out using a cDNA clone as a template and primers of SEQ ID NOS. 24 and 25 on condition that a cycle of reaction steps consisting of 94°C for 1 minute, 60°C for 1 minute and 72°C for 2 minute was repeated 30 times, thereby amplifying the moiety corresponding to the 314th to 1051st of SEQ ID NO. 1. Then, the amplified fragment was cloned into the *EcoRV* site of a vector pBluescript SK(-) (manufactured by Stratagene).

Serving this plasmid DNA as a template and using RNA Transcription Kit (manufactured by Stratagene), transcription was carried out *in vitro* by adding [³⁵S]UTP α S to the reaction solution, thereby making a ³⁵S-labeled single-stranded RNA probe. At this time, an antisense probe was made by using T7RNA polymerase after digesting the plasmid with the restriction endonuclease *EcoRI* (manufactured by Life Technologies) and a sense probe was made by using T3RNA polymerase after digesting the plasmid with *HindIII* (Life Technologies).

The specific reagents and methods for the reaction employed were in accordance with the instructions attached to the kit. The *in situ* hybridization was carried out by somewhat modifying the method given in the literature [*Neuroscience Letters*, 194, 205 (1995)]. That is, the paraffin-embedded specimen was cut into 8 μ m thick sections and placed on silane-coated slide glasses. Incidentally, these procedures were so carried out that ribonuclease might not adhere to the specimen. After removing paraffin, the sections were immersed in 10mM PBS, reacted at 37°C for 10 minutes in 10 μ g/ml of proteinase K and then immersed in 0.1M triethanolamine containing 0.25% (v/v) acetic anhydride for 10 minutes. The section on a formamide paper was covered with 200 μ l of hybridization solution containing approx. 1×10^6 cpm of RNA probe, kept at 65°C for 18 hours, thereby hybridizing to the probe. The section was washed in 2 \times SSC at 65°C for 30 minutes and then in a highly stringent wash (50% formamide, 2 \times SSC, 0.1M DTT) at 65°C for 30 minutes. The section was treated in 1 μ g/ml of ribonuclease A at 37°C for 30 minutes, air-dried, closely adhered to an autoradiographic film b-max (manufactured by Amersham) and then exposed for 48 hours. Next, the section was taken out, immersed in an autoradiographic emulsion [50% (w/v) NR-2 (manufactured by Konica Corporation) distilled water solution], airdried and then exposed at 4°C for 28 days.

As a result, few signals were observed in the case of the sense probe, while high expression was observed in cerebellum, hippocampus and cerebral tonsil as were tissues in which neuronal cell crowded in high density in the case of the antisense probe. On the other hand, white matter where glial cells exist abundantly showed very low expression. In further examination at a microscopic level, high expression was observed in the granule all layer of cerebellum, Purkinje cell of cerebellum, neuronal cell of hippocampus, dentate gyrus, amygdaloid nucleus and cerebral cortex, and the expression was confirmed mainly in nerve cell rather than in glial cells. In addition, the same detection using this antisense probe could

be carried out in regard to young matured rat's brain which had been embedded with paraffin after fixation. The fixation of the brain was carried out by anesthetizing a rat and then perfusing its brain with a 4% paraformaldehyde/10mM phosphate buffer.

Example 6 Acceleration of Apoptosis by Antisense DNA of Human Nap1 Gene

(1) Acceleration of cell death of SH-SY5Y cell by antisense DNA

The SH-SY5Y neuroblastoma cell line was cultured in the same manner as in (2) of Example 5. Human Nap1 antisense phosphorothioate oligodeoxynucleotide (PS-ODN) having a nucleotide sequence represented by SEQ ID NO. 26 and human Nap1 sense PS-ODN having a nucleotide sequence represented by SEQ ID NO. 27 as a control were synthesized (manufactured by Toagosei Chemical Industry Co., Ltd.). Each was mixed with 4 μ l of Lipofect Amine reagent (manufactured by Life Technologies), added to 200 μ l of DMEM and then incubated at room temperature for 30 minutes. Then, 800 μ l of DMEM was added to the incubate, and the resulting mixture was added to the culturing medium of SH-SY5Y cells so as to give a final PS-ODN concentration of 4.5 μ M. Further, the culturing was continued for 12 hours to make the PS-ODN be incorporated in the SH-SY5Y cells. The medium was exchanged for a medium free from PS-ODN, and the culturing was continued further. After 48 hours of medium exchange, many of the cells in which the antisense PS-ODN was incorporated in were changed to have a round cell shape and floated up in the medium, in which a large number of dead cells were observed. On the other hand, absolutely no change was observed in the control cells in which the sense PS-ODN was incorporated.

(2) Acceleration of SH-SY5Y cell apoptosis by antisense DNA

In the same manner as in (1) above, cells were cultured for 12 hours upon adding thereto antisense PS-ODN or sense PS-ODN, thereby making the cells incorporate PS-ODN. After exchanging the medium, the cells were recovered by centrifugation at 1,000g for 5 minutes by every 12 hours in 60 hours. The recovered cells were lysed by adding a cytolysis buffer [50mM Tris-HCl (pH 8.0), 10mM EDTA, 0.3% Triton X-100] thereto and stationarily leaving on ice for 30 minutes. The lysed cells were fractionated into chromatin fractions (precipitate) and DNA fragment fractions (supernatant) by further centrifuging at 27,000g for 20 minutes.

Proteinase K was added to the supernatant at a concentration of 0.5 mg/ml and reacted at 50°C for 1 hour. Ribonuclease A was added to the reaction mixture to give a concentration of 0.4 mg/ml and further reacted at 50°C for 1 hour. Extraction was carried out twice with phenol-chloroform and then precipitated with ethanol, thereby purifying DNA. This DNA was lysed in a TE buffer and electrophoresed on a 1.5% agarose gel. As electrophoresis buffers, 40mM Tris-acetate and 1mM EDTA (pH 7.2) were used. After electrophoresis, DNA was stained with SYBR Green I and subjected to the detection of bands by a UV transilluminator.

The results were shown in Fig. 5. Fig. 5A is an electrophoretogram showing the results of electrophoresis of cell DNA after making the SH-SY5Y neuronal cell line incorporate the antisense PS-ODN (SEQ ID NO. 26). From the left lane of this figure, (m) indicates a DNA size marker (ϕ X174DNA/*Hae*III digestion); (1) indicates SH-SY5Y DNA in case of adding neither reagent nor PS-ODN; (2), (3), (4), (5), (6) and (7) respectively indicate SH-SY5Y DNA after 0 hour, 12 hours, 24 hours, 36 hours, 48 hours and 60 hours from the exchange of medium upon making the cells incorporate the antisense PS-ODN; (8) indicates SH-SY5Y DNA after 60 hours from the exchange of medium upon making the cells incorporate the sense PS-ODN (SEQ ID NO. 27); and (9) indicates SH-SY5Y DNA after 60 hours from the exchange of the medium upon adding a Lipofect Amine reagent alone.

Fig. 5B is an electrophoretogram showing the result of RT-PCR of human Nap1 gene after making the SH-SY5Y neuronal cell line incorporate the antisense PS-ODN (SEQ ID NO. 26). The culturing conditions for the cells in respective lanes are as same as those of Fig. 5A. In Fig. 5B, hNAP 1 shows the result in case of using a human Nap1 gene-specific primer and *ef1 α* shows the result in case of using a primer specific to elongation factor 1 α gene used as a control.

As shown in Fig. 5, DNA ladders characteristic to cells undergone apoptosis were observed in the supernatant of the cells after 48 hours and 60 hours from the exchange of medium upon making the cells incorporate the antisense PS-ODN. This DNA ladder was not observed in the cells after 60 hours from the addition of sense PS-ODN or a Lipofect Amine reagent alone. In addition, when cells made to incorporate antisense PS-ODN were recovered chronologically in the same manner described above and subjected to RT-PCR to human Nap1 gene in the same manner as in Example 2, it was confirmed that the expression of human Nap1 gene mRNA was lowered immediately after 12-hour culturing of the cells upon having added the antisense PS-ODN to make the cells incorporate the same (Fig. 5B).

Besides, with respect to the human Nap1 antisense PS-ODN represented by SEQ ID NO. 28 (manufactured by Toagosei Chemical Industry Co., Ltd.) corresponding to another site of the Nap1 gene and the control sense PS-ODN represented by SEQ ID NO. 29 (Toagosei Chemical Industry Co., Ltd.), DNAs of the SH-SY5Y cells, in which the above nucleotides were incorporated, after 60 hours from the change of media were electrophoretically examined similarly. As a result, DNA ladders characteristic to cells undergone apoptosis were observed in the supernatant of the cells in which antisense PS-ODN had been incorporated, while no DNA ladder was observed in the sense PS-ODN, either.

Therefore, it was found that the abundance of Nap1 gene was lowered to cause apoptosis of the SH-SY5Y cells by making the cells incorporate the antisense PS-ODN. From this, it is considered that the Nap gene suppress the apoptosis of cells.

Besides, the detection of apoptosis by *in situ* end label assay [*Journal of Cellular Biology*, 119, 493 (1992)] was also carried out. The SH-SY5Y cells were sprayed on a cover glass and cultured for 48 hours in the same medium as that of (3) of Example 5. After adding the antisense PS-ODN (SEQ ID NO. 26) or the control sense PS-ODN (SEQ ID NO.

27) to the medium, the medium was incubated for 12 hours to make the cells incorporate the PS-ODN, exchanged for a medium free from PS-ODN and further incubated for 24 hours. With respect to this cell, the detection of apoptosis was carried out using TACS In situ Apoptosis Detection Kit (manufactured by Trevigen). The specific reagents and methods employed were in accordance with the instructions of the kit. DNAs in the nucleosome digested by apoptosis were end-specifically labeled with Biotin, bound with peroxidase-labeled streptoavidin and then reacted using TBL as a peroxidase chromogenic reagent. As a result, deep blue was produced and apoptosis was detected in the cells in which the antisense PS-ODN had been incorporated, while apoptosis was not detected in the cells in which the control sense PS-ODN was incorporated.

(3) Acceleration of apoptosis by antisense DNAs in LA-N-5 cells and retinoic acid-treated SH-SY5Y cells

Whether or not Nap1 gene antisense DNA causes apoptosis even in neuronal cells which are more differentiated than the SH-SY5Y cells was examined. That is, the SH-SY5Y cells cultured by adding thereto 10 μ M all trans retinoic acid (RA) (manufactured by Sigma) and the LA-N-5 neuroblastoma cell line which is considered to be a little more differentiated than the SH-SY5Y cell were subjected to an experiment for making them incorporate the antisense PS-ODN represented by SEQ ID NO. 26 similarly to (2) above. Incidentally, it is said that the SH-SY5Y cell so differentiates as to have properties of a matured nerve cell such that, by the treatment with 10 μ M RA, it elongates neurites, it discontinues proliferation, its cell membrane generates an action potential and so on.

Also in the case of the 10 μ M RA-treated SH-SY5Y cells and the LA-N-5 cells, DNA ladders characteristic to cells undergone apoptosis were observed in the cells in which the antisense PS-ODN had been incorporated, but were not observed in the cells in which the sense PS-ODN had been incorporated. Therefore, it was confirmed that the Nap1 gene antisense DNA caused apoptosis even in a more differentiated neurocyte.

Besides, in the same manner described in (2) above, the detection of apoptosis by *in situ* end label assay was carried out in regard to LA-N-5 cells. As a result, apoptosis was detected in the antisense PS-ODN but not in the control sense PS-ODN similarly.

[Effect of the Invention]

According to the present invention, a human Nap1 protein, a DNA encoding said protein, a process for producing said protein, an antibody recognizing said protein and the uses of said protein are provided.

By the use of a novel gene to be obtained according to the present invention, the treatment and the diagnosis of Alzheimer's disease become possible.

[Sequence Listing]

SEQ ID NO: 1

LENGTH: 4236

TYPE: nucleic acid

NUMBER OF STRANDS: DOUBLE

TOPOLOGY: linear

MOLECULAR TYPE: cDNA to mRNA

ORIGINAL SOURCE:

ORGANISM: Human (Homo sapiens)

TYPE OF TISSUE: brain

FEATURE:

NAME/KEY: CDS

LOCATION: 20..3406

SEQUENCE DESCRIPTION:

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CGGCGGCACC AGCACCACC ATG TCG CGC TCA GTG CTG CAG CCC AGT CAG CAG      52
                               Met Ser Arg Ser Val Leu Gln Pro Ser Gln Gln
                               1           5           10
AAG CTG GCG GAG AAG CTC ACC ATC CTC AAC GAC CGG GGC GTC GGC ATG      100
Lys Leu Ala Glu Lys Leu Thr Ile Leu Asn Asp Arg Gly Val Gly Met
                               15           20           25
CTC ACC CGC CTC TAC AAC ATC AAG AAG GCA TGT GGA GAC CCC AAG GCA      148
Leu Thr Arg Leu Tyr Asn Ile Lys Lys Ala Cys Gly Asp Pro Lys Ala
                               30           35           40
AAA CCA TCC TAT CTT ATC GAC AAA AAC CTG GAA TCT GCT GTG AAA TTC      196
Lys Pro Ser Tyr Leu Ile Asp Lys Asn Leu Glu Ser Ala Val Lys Phe
                               45           50           55
ATA GTC AGA AAA TTC CCT GCT GTA GAA ACC CGC AAC AAC AAT CAA CAG      244
Ile Val Arg Lys Phe Pro Ala Val Glu Thr Arg Asn Asn Asn Gln Gln
                               60           65           70           75
CTT GCA CAA CTA CAG AAA GAA AAA TCA GAG ATT CTG AAA AAT CTG GCA      292
Leu Ala Gln Leu Gln Lys Glu Lys Ser Glu Ile Leu Lys Asn Leu Ala
                               80           85           90
TTA TAT TAC TTC ACA TTT GTA GAT GTT ATG GAA TTT AAG GAC CAT GTT      340
Leu Tyr Tyr Phe Thr Phe Val Asp Val Met Glu Phe Lys Asp His Val
                               95           100           105
TGT GAA TTG CTG AAT ACT ATT GAC GTT TGC CAA GTC TTC TTT GAT ATT      388
Cys Glu Leu Leu Asn Thr Ile Asp Val Cys Gln Val Phe Phe Asp Ile
                               110           115           120
ACT GTA AAC TTT GAT TTA ACA AAG AAC TAC TTA GAT TTA ATT ATA ACC      436
Thr Val Asn Phe Asp Leu Thr Lys Asn Tyr Leu Asp Leu Ile Ile Thr
                               125           130           135
TAT ACA ACA CTA ATG ATA CTG CTG TCT CGA ATT GAA GAA AGG AAG GCA      484
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Tyr Thr Thr Leu Met Ile Leu Leu Ser Arg Ile Glu Glu Arg Lys Ala	
140	155
ATC ATT GGA TTA TAC AAC TAT GCC CAT GAA ATG ACT CAT GGA GCA AGT	532
Ile Ile Gly Leu Tyr Asn Tyr Ala His Glu Met Thr His Gly Ala Ser	
160	170
GAC AGA GAA TAC CCA CGC CTT GGC CAG ATG ATT GTG GAT TAT GAA AAC	580
Asp Arg Glu Tyr Pro Arg Leu Gly Gln Met Ile Val Asp Tyr Glu Asn	
175	185
CCT TTA AAG AAG ATG ATG GAA GAA TTT GTA CCC CAT AGC AAG TCT CTT	628
Pro Leu Lys Lys Met Met Glu Glu Phe Val Pro His Ser Lys Ser Leu	
190	200
TCA GAT GCA CTA ATT TCT CTT CAA ATG GTA TAT CCT CGA AGG AAT CTT	676
Ser Asp Ala Leu Ile Ser Leu Gln Met Val Tyr Pro Arg Arg Asn Leu	
205	215
TCA GCT GAC CAG TGG AGA AAT GCC CAG TTA TTG AGC CTC ATC AGT GCA	724
Ser Ala Asp Gln Trp Arg Asn Ala Gln Leu Leu Ser Leu Ile Ser Ala	
220	235
CCT AGT ACA ATG CTT AAT CCA GCA CAG TCC GAC ACT ATG CCT TGT GAA	772
Pro Ser Thr Met Leu Asn Pro Ala Gln Ser Asp Thr Met Pro Cys Glu	
240	250
TAC CTC TCT TTG GAT GCA ATG GAA AAG TGG ATT ATC TTT GGC TTT ATT	820
Tyr Leu Ser Leu Asp Ala Met Glu Lys Trp Ile Ile Phe Gly Phe Ile	
255	265
TTG TGC CAT GGG ATC CTA AAT ACT GAC GCT ACA GCA CTG AAC CTT TGG	868
Leu Cys His Gly Ile Leu Asn Thr Asp Ala Thr Ala Leu Asn Leu Trp	
270	280
AAA CTA GCT CTT CAA AGT AGC TCT TGC CTC TCT CTC TTT CGG GAT GAA	916
Lys Leu Ala Leu Gln Ser Ser Ser Cys Leu Ser Leu Phe Arg Asp Glu	
285	295
GTT TTC CAC ATT CAC AAA GCT GCA GAA GAC TTA TTT GTA AAC ATA CGA	964
Val Phe His Ile His Lys Ala Ala Glu Asp Leu Phe Val Asn Ile Arg	
300	315
GGC TAT AAT AAA CGT ATT AAT GAC ATA AGA GAA TGC AAG GAG GCA GCC	1012
Gly Tyr Asn Lys Arg Ile Asn Asp Ile Arg Glu Cys Lys Glu Ala Ala	
320	330
GTG TCA CAT GCT GGT TCA ATG CAC AGA GAA AGA CGC AAG TTT TTA AGA	1060
Val Ser His Ala Gly Ser Met His Arg Glu Arg Arg Lys Phe Leu Arg	
335	345
TCT GCA CTG AAG GAA TTG GCT ACT GTC CTC TCT GAT CAA CCT GGA TTG	1108
Ser Ala Leu Lys Glu Leu Ala Thr Val Leu Ser Asp Gln Pro Gly Leu	

350	355	360	
CTA GGT CCC AAG GCA CTT TTT GTT TTT ATG GCA TTA TCC TTT GCC CGT			1156
Leu Gly Pro Lys Ala Leu Phe Val Phe Met Ala Leu Ser Phe Ala Arg			
365	370	375	
GAT GAA ATC ATC TGG CTA CTT CGT CAT GCA GAT AAC ATG CCA AAG AAG			1204
Asp Glu Ile Ile Trp Leu Leu Arg His Ala Asp Asn Met Pro Lys Lys			
380	385	390	395
AGT GCA GAC GAC TTT ATA GAT AAG CAC ATT GCT GAA TTA ATA TTT TAC			1252
Ser Ala Asp Asp Phe Ile Asp Lys His Ile Ala Glu Leu Ile Phe Tyr			
400	405		410
ATG GAA GAA CTT AGA GCA CAT GTG AGG AAA TAC GGA CCT GTA ATG CAG			1300
Met Glu Glu Leu Arg Ala His Val Arg Lys Tyr Gly Pro Val Met Gln			
415	420	425	
AGG TAT TAC GTG CAG TAC CTT TCT GGC TTT GAT GCT GTT GTC CTC AAT			1348
Arg Tyr Tyr Val Gln Tyr Leu Ser Gly Phe Asp Ala Val Val Leu Asn			
430	435	440	
GAA CTC GTG CAG AAT CTT TCT GTT TGC CCT GAA GAT GAA TCA ATC ATC			1396
Glu Leu Val Gln Asn Leu Ser Val Cys Pro Glu Asp Glu Ser Ile Ile			
445	450	455	
ATG TCC TCT TTT GTT AAC ACT ATG ACT TCC CTA AGT GTA AAA CAA GTT			1444
Met Ser Ser Phe Val Asn Thr Met Thr Ser Leu Ser Val Lys Gln Val			
460	465	470	475
GAA GAT GGG GAA GTA TTT GAT TTC AGA GGA ATG AGA TTA GAT TGG TTT			1492
Glu Asp Gly Glu Val Phe Asp Phe Arg Gly Met Arg Leu Asp Trp Phe			
480	485		490
AGG TTA CAG GCA TAT ACT AGT GTC TCA AAG GCT TCA CTT GGC CTT GCA			1540
Arg Leu Gln Ala Tyr Thr Ser Val Ser Lys Ala Ser Leu Gly Leu Ala			
495	500	505	
GAT CAC AGA GAA CTT GGG AAG ATG ATG AAT ACA ATA ATT TTT CAT ACA			1588
Asp His Arg Glu Leu Gly Lys Met Met Asn Thr Ile Ile Phe His Thr			
510	515	520	
AAA ATG GTA GAT TCC TTG GTG GAA ATG TTG GTG GAA ACA TCA GAT CTC			1636
Lys Met Val Asp Ser Leu Val Glu Met Leu Val Glu Thr Ser Asp Leu			
525	530	535	
TCC ATA TTT TGT TTT TAT AGT CGT GCT TTT GAG AAG ATG TTT CAA CAG			1684
Ser Ile Phe Cys Phe Tyr Ser Arg Ala Phe Glu Lys Met Phe Gln Gln			
540	545	550	555
TGT TTG GAG TTA CCC TCT CAA TCA AGA TAC TCA ATT GCA TTT CCA CTA			1732
Cys Leu Glu Leu Pro Ser Gln Ser Arg Tyr Ser Ile Ala Phe Pro Leu			
560	565		570

CTT TGC ACT CAT TTT ATG AGT TGC ACG CAT GAA CTA TGT CCA GAA GAG	1780
Leu Cys Thr His Phe Met Ser Cys Thr His Glu Leu Cys Pro Glu Glu	
575 580 585	
CGA CAT CAT ATT GGA GAT CGC AGT CTT TCC TTA TGT AAT ATG TTC CTA	1828
Arg His His Ile Gly Asp Arg Ser Leu Ser Leu Cys Asn Met Phe Leu	
590 595 600	
GAT GAA ATG GCC AAA CAA GCT CGA AAT CTC ATC ACT GAT ATT TGC ACA	1876
Asp Glu Met Ala Lys Gln Ala Arg Asn Leu Ile Thr Asp Ile Cys Thr	
605 610 615	
GAA CAG TGT ACC CTT AGT GAC CAG TTG CTA CCC AAG CAT TGT GCC AAA	1924
Glu Gln Cys Thr Leu Ser Asp Gln Leu Leu Pro Lys His Cys Ala Lys	
620 625 630 635	
ACT ATC AGT CAA GCA GTG AAT AAG AAA TCA AAA AAG CAG ACT GGT AAG	1972
Thr Ile Ser Gln Ala Val Asn Lys Lys Ser Lys Lys Gln Thr Gly Lys	
640 645 650	
AAA GGG GAA CCT GAA AGG GAG AAA CCA GGT GTT GAG AGC ATG AGG AAA	2020
Lys Gly Glu Pro Glu Arg Glu Lys Pro Gly Val Glu Ser Met Arg Lys	
655 660 665	
AAC AGG CTG GTT GTG ACC AAC CTT GAT AAA TTG CAC ACT GCA CTT TCT	2068
Asn Arg Leu Val Val Thr Asn Leu Asp Lys Leu His Thr Ala Leu Ser	
670 675 680	
GAG TTA TGC TTC TCT ATA AAT TAT GTA CCA AAC ATG GTG GTA TGG GAA	2116
Glu Leu Cys Phe Ser Ile Asn Tyr Val Pro Asn Met Val Val Trp Glu	
685 690 695	
CAT ACC TTT ACC CCA CGA GAA TAT TTG ACT TCT CAT CTG GAA ATA CGC	2164
His Thr Phe Thr Pro Arg Glu Tyr Leu Thr Ser His Leu Glu Ile Arg	
700 705 710 715	
TTT ACC AAG TCA ATT GTT GGG ATG ACT ATG TAT AAT CAA GCC ACA CAG	2212
Phe Thr Lys Ser Ile Val Gly Met Thr Met Tyr Asn Gln Ala Thr Gln	
720 725 730	
GAA ATT GCA AAA CCT TCA GAA CTT CTA ACA AGT GTA AGA GCA TAC ATG	2260
Glu Ile Ala Lys Pro Ser Glu Leu Leu Thr Ser Val Arg Ala Tyr Met	
735 740 745	
ACC GTA CTC CAG TCA ATA GAA AAC TAT GTG CAG ATT GAT ATT ACA AGA	2308
Thr Val Leu Gln Ser Ile Glu Asn Tyr Val Gln Ile Asp Ile Thr Arg	
750 755 760	
GTA TTT AAT AAT GTG CTT CTT CAA CAA ACA CAA CAT TTA GAC AGT CAT	2356
Val Phe Asn Asn Val Leu Leu Gln Gln Thr Gln His Leu Asp Ser His	
765 770 775	
GGA GAG CCA ACC ATT ACA AGT CTA TAC ACA AAT TGG TAT TTG GAA ACT	2404

Gly	Glu	Pro	Thr	Ile	Thr	Ser	Leu	Tyr	Thr	Asn	Trp	Tyr	Leu	Glu	Thr		
780						785					790						795
TTG	TTA	CGA	CAA	GTC	AGC	AAT	GGC	CAT	ATA	GCA	TAT	TTT	CCT	GCA	ATG		2452
Leu	Leu	Arg	Gln	Val	Ser	Asn	Gly	His	Ile	Ala	Tyr	Phe	Pro	Ala	Met		
					800						805						810
AAA	GCG	TTT	GTG	AAC	TTA	CCT	ACA	GAA	AAT	GAA	TTA	ACA	TTC	AAT	GCA		2500
Lys	Ala	Phe	Val	Asn	Leu	Pro	Thr	Glu	Asn	Glu	Leu	Thr	Phe	Asn	Ala		
				815						820					825		
GAG	GAA	TAT	TCT	GAC	ATA	TCA	GAA	ATG	AGG	TCA	TTA	TCA	GAA	CTA	CTA		2548
Glu	Glu	Tyr	Ser	Asp	Ile	Ser	Glu	Met	Arg	Ser	Leu	Ser	Glu	Leu	Leu		
				830					835					840			
GGC	CCA	TAT	GGT	ATG	AAG	TTT	CTA	AGT	GAA	AGC	CTT	ATG	TGG	CAT	ATT		2596
Gly	Pro	Tyr	Gly	Met	Lys	Phe	Leu	Ser	Glu	Ser	Leu	Met	Trp	His	Ile		
				845			850						855				
TCA	TCA	CAA	GTT	GCT	GAA	CTT	AAG	AAA	CTT	GTG	GTG	GAG	AAT	GTT	GAT		2644
Ser	Ser	Gln	Val	Ala	Glu	Leu	Lys	Lys	Leu	Val	Val	Glu	Asn	Val	Asp		
860						865						870					875
GTG	TTA	ACA	CAA	ATG	AGG	ACC	AGC	TTT	GAC	AAA	CCA	GAC	CAG	ATG	GCT		2692
Val	Leu	Thr	Gln	Met	Arg	Thr	Ser	Phe	Asp	Lys	Pro	Asp	Gln	Met	Ala		
					880						885				890		
GCA	CTG	TTT	AAA	AGA	TTA	TCA	TCT	GTT	GAC	AGT	GTC	TTG	AAG	AGG	ATG		2740
Ala	Leu	Phe	Lys	Arg	Leu	Ser	Ser	Val	Asp	Ser	Val	Leu	Lys	Arg	Met		
				895					900					905			
ACA	ATA	ATT	GGT	GTA	ATT	TTA	TCC	TTC	CGA	TCA	TTG	GCA	CAA	GAA	GCA		2788
Thr	Ile	Ile	Gly	Val	Ile	Leu	Ser	Phe	Arg	Ser	Leu	Ala	Gln	Glu	Ala		
			910					915						920			
CTT	AGA	GAT	GTG	TTA	TCC	TAC	CAC	ATT	CCT	TTT	CTT	GTA	AGT	TCA	ATT		2836
Leu	Arg	Asp	Val	Leu	Ser	Tyr	His	Ile	Pro	Phe	Leu	Val	Ser	Ser	Ile		
		925				930						935					
GAA	GAT	TTT	AAG	GAT	CAC	ATT	CCA	AGG	GAA	ACT	GAT	ATG	AAG	GTT	GCA		2884
Glu	Asp	Phe	Lys	Asp	His	Ile	Pro	Arg	Glu	Thr	Asp	Met	Lys	Val	Ala		
940					945						950						955
ATG	AAT	GTG	TAT	GAG	TTA	TCA	TCA	GCT	GCC	GGA	TTA	CCT	TGT	GAG	ATT		2932
Met	Asn	Val	Tyr	Glu	Leu	Ser	Ser	Ala	Ala	Gly	Leu	Pro	Cys	Glu	Ile		
					960						965				970		
GAT	CCT	GCA	TTG	GTC	GTA	GCT	CTT	TCT	TCA	CAA	AAA	TCG	GAA	AAC	ATT		2980
Asp	Pro	Ala	Leu	Val	Val	Ala	Leu	Ser	Ser	Gln	Lys	Ser	Glu	Asn	Ile		
				975					980					985			
AGT	CCA	GAA	GAA	GAG	TAT	AAA	ATT	GCC	TGC	CTT	CTC	ATG	GTG	TTT	GTG		3028
Ser	Pro	Glu	Glu	Glu	Tyr	Lys	Ile	Ala	Cys	Leu	Leu	Met	Val	Phe	Val		

990	995	1000	
GCA GTT TCT TTG CCA ACA CTG GCC AGT AAT GTG ATG TCT CAG TAC AGC			3076
Ala Val Ser Leu Pro Thr Leu Ala Ser Asn Val Met Ser Gln Tyr Ser			
1005	1010	1015	
CCT GCT ATA GAA GGG CAT TGC AAC AAC ATA CAT TGC TTG GCC AAA GCC			3124
Pro Ala Ile Glu Gly His Cys Asn Asn Ile His Cys Leu Ala Lys Ala			
1020	1025	1030	1035
ATC AAC CAG ATT GCT GCA GCT TTG TTT ACA ATT CAC AAA GGA AGC ATT			3172
Ile Asn Gln Ile Ala Ala Ala Leu Phe Thr Ile His Lys Gly Ser Ile			
1040	1045	1050	
GAA GAC CGT CTT AAA GAA TTT CTG GCG CTT GCA TCC TCC AGT CTA CTG			3220
Glu Asp Arg Leu Lys Glu Phe Leu Ala Leu Ala Ser Ser Ser Leu Leu			
1055	1060	1065	
AAA ATT GGC CAG GAG ACA GAT AAA ACT ACA ACA AGA AAT AGA GAA TCT			3268
Lys Ile Gly Gln Glu Thr Asp Lys Thr Thr Thr Arg Asn Arg Glu Ser			
1070	1075	1080	
GTT TAT TTA CTG CTA GAT ATG ATT GTA CAA GAA TCT CCA TTC CTT ACA			3316
Val Tyr Leu Leu Leu Asp Met Ile Val Gln Glu Ser Pro Phe Leu Thr			
1085	1090	1095	
ATG GAT CTT TTG GAA TCT TGT TTT CCT TAT GTC TTG CTG AGA AAT GCA			3364
Met Asp Leu Leu Glu Ser Cys Phe Pro Tyr Val Leu Leu Arg Asn Ala			
1100	1105	1110	1115
TAC CAT GCT GTC TAC AAA CAA AGT GTT ACA TCT TCT GCA TAAAATTACC			3413
Tyr His Ala Val Tyr Lys Gln Ser Val Thr Ser Ser Ala			
1120	1125		
TACTTAATCA AGATAAGCAC GCATTTTTGT TGCCTTGGTT TTACCTGTAG ACTGTGGAAC			3473
TATTTTACCT TAAGACCTGA AAAAGTTTTG TGGATTATAA ATTTCTTTCA TACGGTTGTA			3533
TTTTCTGATC ATTGGTTTCT TAATATGGTT GTACTACAGT ATACTTGGTT GATTTAGGTT			3593
GCACATTCAC TGAATTCAC TGAATTATTC CTATAATTTT AAAGTATCAT TTATTTGAAA			3653
AACATACATT ATCAACATGT TTTTGATATT TGATAATGAA AAAAATCTTT GCTTGTTTAT			3713
TTCTGAAAAA GAACTGTATT TAGTGATTAT TTTAGATAGT GATATTATAG CATTATCTG			3773
TGTGTAAATT ATTTTCATATA GGGAAGAGTT CTGATCTGTA CCTATGGTTC TTATTGAAAA			3833
CAACATTGGA TGTGCATTTC TGTGATGTTA TGAATACATT TCTACTTTAT TTTGAAACAT			3893
TTGCCAAACT AAATACTGTA AACTGTATA ACATTTAAAA ATGTTAAAGA ACTGCTTAGT			3953
ATTAGAAGCA GATCATTTCC CAAAATTCTA AGAGCAGCAG CATATGTTGT TGCTTGATA			4013
AAGCCTAGCG ATAATTTTAA GACTAACTTC CATGGTGCCC TGTTGGCATT AGCACTACCA			4073
TTGTACCCTG CTGTATAATA AACAATCTTA GACATTTATC AACTGTTGAT ACAAATGTTA			4133
GTCCCTAACC ACTTTTTATA TATGTTTTAA ATTTTGTAAA TTCAAGTGTA CCTTCCATAA			4193
CATAAAATAA AACTAGACT GTATCAAAAA AAAAAAAAAA AAA			4236

SEQ ID NO: 2

LENGTH: 1128

TYPE: amino acid

TOPOLOGY: linear

MOLECULAR TYPE: protein

ORIGINAL SOURCE:

ORGANISM: Human (Homo sapiens)

TYPE OF TISSUE: brain

SEQUENCE DESCRIPTION:

Met	Ser	Arg	Ser	Val	Leu	Gln	Pro	Ser	Gln	Gln	Lys	Leu	Ala	Glu	Lys		
1					5						10						15
Leu	Thr	Ile	Leu	Asn	Asp	Arg	Gly	Val	Gly	Met	Leu	Thr	Arg	Leu	Tyr		
				20						25					30		
Asn	Ile	Lys	Lys	Ala	Cys	Gly	Asp	Pro	Lys	Ala	Lys	Pro	Ser	Tyr	Leu		
			35						40					45			
Ile	Asp	Lys	Asn	Leu	Glu	Ser	Ala	Val	Lys	Phe	Ile	Val	Arg	Lys	Phe		
			50					55					60				
Pro	Ala	Val	Glu	Thr	Arg	Asn	Asn	Asn	Gln	Gln	Leu	Ala	Gln	Leu	Gln		
65						70					75						80
Lys	Glu	Lys	Ser	Glu	Ile	Leu	Lys	Asn	Leu	Ala	Leu	Tyr	Tyr	Phe	Thr		
						85					90						95
Phe	Val	Asp	Val	Met	Glu	Phe	Lys	Asp	His	Val	Cys	Glu	Leu	Leu	Asn		
				100					105					110			
Thr	Ile	Asp	Val	Cys	Gln	Val	Phe	Phe	Asp	Ile	Thr	Val	Asn	Phe	Asp		
			115						120					125			
Leu	Thr	Lys	Asn	Tyr	Leu	Asp	Leu	Ile	Ile	Thr	Tyr	Thr	Thr	Leu	Met		
			130				135						140				
Ile	Leu	Leu	Ser	Arg	Ile	Glu	Glu	Arg	Lys	Ala	Ile	Ile	Gly	Leu	Tyr		
145					150					155							160
Asn	Tyr	Ala	His	Glu	Met	Thr	His	Gly	Ala	Ser	Asp	Arg	Glu	Tyr	Pro		
					165					170							175
Arg	Leu	Gly	Gln	Met	Ile	Val	Asp	Tyr	Glu	Asn	Pro	Leu	Lys	Lys	Met		
				180						185				190			
Met	Glu	Glu	Phe	Val	Pro	His	Ser	Lys	Ser	Leu	Ala	Asp	Ala	Leu	Ile		
			195						200					205			
Ser	Leu	Gln	Met	Val	Tyr	Pro	Arg	Arg	Asn	Leu	Ser	Ala	Asp	Gln	Trp		
			210					215						220			
Arg	Asn	Ala	Gln	Leu	Leu	Ser	Leu	Ile	Ser	Ala	Pro	Ser	Thr	Met	Leu		
225					230					235							240
Asn	Pro	Ala	Gln	Ser	Asp	Thr	Met	Pro	Cys	Glu	Tyr	Leu	Ser	Leu	Asp		

	245	250	255
Ala Met Glu Lys Trp Ile Ile Phe Gly Phe Ile Leu Cys His Gly Ile			
	260	265	270
Leu Asn Thr Asp Ala Thr Ala Leu Asn Leu Trp Lys Leu Ala Leu Gln			
	275	280	285
Ser Ser Ser Cys Leu Ser Leu Phe Arg Asp Glu Val Phe His Ile His			
	290	295	300
Lys Ala Ala Glu Asp Leu Phe Val Asn Ile Arg Gly Tyr Asn Lys Arg			
305	310	315	320
Ile Asn Asp Ile Arg Glu Cys Lys Glu Ala Ala Val Ser His Ala Gly			
	325	330	335
Ser Met His Arg Glu Arg Arg Lys Phe Leu Arg Ser Ala Leu Lys Glu			
	340	345	350
Leu Ala Thr Val Leu Ser Asp Gln Pro Gly Leu Leu Gly Pro Lys Ala			
	355	360	365
Leu Phe Val Phe Met Ala Leu Ser Phe Ala Arg Asp Glu Ile Ile Trp			
	370	375	380
Leu Leu Arg His Ala Asp Asn Met Pro Lys Lys Ser Ala Asp Asp Phe			
385	390	395	400
Ile Asp Lys His Ile Ala Glu Leu Ile Phe Tyr Met Glu Glu Leu Arg			
	405	410	415
Ala His Val Arg Lys Tyr Gly Pro Val Met Gln Arg Tyr Tyr Val Gln			
	420	425	430
Tyr Leu Ser Gly Phe Asp Ala Val Val Leu Asn Glu Leu Val Gln Asn			
	435	440	445
Leu Ser Val Cys Pro Glu Asp Glu Ser Ile Ile Met Ser Ser Phe Val			
	450	455	460
Asn Thr Met Thr Ser Leu Ser Val Lys Gln Val Glu Asp Gly Glu Val			
465	470	475	480
Phe Asp Phe Arg Gly Met Arg Leu Asp Trp Phe Arg Leu Gln Ala Tyr			
	485	490	495
Thr Ser Val Ser Lys Ala Ser Leu Gly Leu Ala Asp His Arg Glu Leu			
	500	505	510
Gly Lys Met Met Asn Thr Ile Ile Phe His Thr Lys Met Val Asp Ser			
	515	520	525
Leu Val Glu Met Leu Val Glu Thr Ser Asp Leu Ser Ile Phe Cys Phe			
	530	535	540
Tyr Ser Arg Ala Phe Glu Lys Met Phe Gln Gln Cys Leu Glu Leu Pro			
545	550	555	560
Ser Gln Ser Arg Tyr Ser Ile Ala Phe Pro Leu Leu Cys Thr His Phe			

	565		570		575
Met Ser Cys Thr His Glu Leu Cys Pro Glu Glu Arg His His Ile Gly					
	580		585		590
Asp Arg Ser Leu Ser Leu Cys Asn Met Phe Leu Asp Glu Met Ala Lys					
	595		600		605
Gln Ala Arg Asn Leu Ile Thr Asp Ile Cys Thr Glu Gln Cys Thr Leu					
	610		615		620
Ser Asp Gln Leu Leu Pro Lys His Cys Ala Lys Thr Ile Ser Gln Ala					
625		630		635	640
Val Asn Lys Lys Ser Lys Lys Gln Thr Gly Lys Lys Gly Glu Pro Glu					
	645		650		655
Arg Glu Lys Pro Gly Val Glu Ser Met Arg Lys Asn Arg Leu Val Val					
	660		665		670
Thr Asn Leu Asp Lys Leu His Thr Ala Leu Ser Glu Leu Cys Phe Ser					
	675		680		685
Ile Asn Tyr Val Pro Asn Met Val Val Trp Glu His Thr Phe Thr Pro					
	690		695		700
Arg Glu Tyr Leu Thr Ser His Leu Glu Ile Arg Phe Thr Lys Ser Ile					
705		710		715	720
Val Gly Met Thr Met Tyr Asn Gln Ala Thr Gln Glu Ile Ala Lys Pro					
	725		730		735
Ser Glu Leu Leu Thr Ser Val Arg Ala Tyr Met Thr Val Leu Gln Ser					
	740		745		750
Ile Glu Asn Tyr Val Gln Ile Asp Ile Thr Arg Val Phe Asn Asn Val					
	755		760		765
Leu Leu Gln Gln Thr Gln His Leu Asp Ser His Gly Glu Pro Thr Ile					
	770		775		780
Thr Ser Leu Tyr Thr Asn Trp Tyr Leu Glu Thr Leu Leu Arg Gln Val					
785		790		795	800
Ser Asn Gly His Ile Ala Tyr Phe Pro Ala Met Lys Ala Phe Val Asn					
	805		810		815
Leu Pro Thr Glu Asn Glu Leu Thr Phe Asn Ala Glu Glu Tyr Ser Asp					
	820		825		830
Ile Ser Glu Met Arg Ser Leu Ser Glu Leu Leu Gly Pro Tyr Gly Met					
	835		840		845
Lys Phe Leu Ser Glu Ser Leu Met Trp His Ile Ser Ser Gln Val Ala					
	850		855		860
Glu Leu Lys Lys Leu Val Val Glu Asn Val Asp Val Leu Thr Gln Met					
865		870		875	880
Arg Thr Ser Phe Asp Lys Pro Asp Gln Met Ala Ala Leu Phe Lys Arg					

	885		890		895
Leu	Ser	Ser	Val	Asp	Ser
		Val	Leu	Lys	Arg
			Met	Thr	Ile
				Ile	Gly
				Val	
	900		905		910
Ile	Leu	Ser	Phe	Arg	Ser
		Leu	Ala	Gln	Glu
			Ala	Leu	Arg
				Asp	Val
				Leu	
	915		920		925
Ser	Tyr	His	Ile	Pro	Phe
		Leu	Val	Ser	Ser
				Ile	Glu
				Asp	Phe
				Lys	Asp
	930		935		940
His	Ile	Pro	Arg	Glu	Thr
		Asp	Met	Lys	Val
			Ala	Met	Asn
				Val	Tyr
				Glu	
945		950		955	
					960
Leu	Ser	Ser	Ala	Ala	Gly
		Leu	Pro	Cys	Glu
				Ile	Asp
				Pro	Ala
				Leu	Val
	965		970		975
Val	Ala	Leu	Ser	Ser	Gln
		Lys	Ser	Glu	Asn
			Ile	Ser	Pro
				Glu	Glu
				Glu	
	980		985		990
Tyr	Lys	Ile	Ala	Cys	Leu
		Leu	Met	Val	Phe
				Val	Ala
				Val	Ser
				Leu	Pro
	995		1000		1005
Thr	Leu	Ala	Ser	Asn	Val
		Met	Ser	Gln	Tyr
				Ser	Pro
				Ala	Ile
				Glu	Gly
	1010		1015		1020
His	Cys	Asn	Asn	Ile	His
		Cys	Leu	Ala	Lys
			Ala	Ile	Asn
				Gln	Ile
				Ala	
1025		1030		1035	
					1040
Ala	Ala	Leu	Phe	Thr	Ile
		His	Lys	Gly	Ser
				Ile	Glu
				Asp	Arg
				Leu	Lys
	1045		1050		1055
Glu	Phe	Leu	Ala	Leu	Ala
		Ser	Ser	Ser	Leu
				Lys	Ile
				Gly	Gln
				Glu	
	1060		1065		1070
Thr	Asp	Lys	Thr	Thr	Thr
		Arg	Asn	Arg	Glu
				Ser	Val
				Tyr	Leu
				Leu	Leu
				Leu	
	1075		1080		1085
Asp	Met	Ile	Val	Gln	Glu
		Ser	Pro	Phe	Leu
				Thr	Met
				Asp	Leu
				Leu	Glu
	1090		1095		1100
Ser	Cys	Phe	Pro	Tyr	Val
		Leu	Leu	Arg	Asn
				Ala	Tyr
				His	Ala
				Val	Tyr
1105		1110		1115	
					1120
Lys	Gln	Ser	Val	Thr	Ser
		Ser	Ala		
	1125				

SEQ ID NO: 3

LENGTH: 265

TYPE: nucleic acid

NUMBER OF STRANDS: DOUBLE

TOPOLOGY: linear

MOLECULAR TYPE: cDNA to mRNA

ORIGINAL SOURCE:

ORGANISM: Human (Homo sapiens)

TYPE OF TISSUE: brain

CAGCAGCAGC	AGCAGCATAT	GTGTTGCTT	GTATAAGCC	TAGCGATAAT	TTTtagacta	60
ACTTCCATGG	TGCCCTGTTG	GCATTAGCAC	TACCATTGTA	CCCTGCTGTA	TAATAAACAA	120
TCTTAGACAT	TTATCAACTG	TTGATACAAA	TGTTAGTCCC	TAACCACGTT	TTATATATGT	180
TTTAAATTTT	TGAAATTCAA	GTGTACCTTC	CATAACATAA	AATAAACACT	AGACTGTATC	240
AAAAAAAAAA	AAAAAAAAAA	AAAAA				

SEQ ID NO: 4
 LENGTH: 26
 TYPE: nucleic acid
 NUMBER OF STRANDS: SINGLE
 TOPOLOGY: linear
 MOLECULAR TYPE: other nucleic acid, synthetic DNA
 TTTTTTTTT TTTTTTTTT TTTTTTG

16

SEQ ID NO: 5
 LENGTH: 26
 TYPE: nucleic acid
 NUMBER OF STRANDS: SINGLE
 TOPOLOGY: linear
 MOLECULAR TYPE: other nucleic acid, synthetic DNA
 TTTTTTTTT TTTTTTTTT TTTTTTA

16

SEQ ID NO: 6
 LENGTH: 26
 TYPE: nucleic acid
 NUMBER OF STRANDS: SINGLE
 TOPOLOGY: linear
 MOLECULAR TYPE: other nucleic acid, synthetic DNA
 TTTTTTTTT TTTTTTTTT TTTTTTC

16

SEQ ID NO: 7
 LENGTH: 15
 TYPE: nucleic acid
 NUMBER OF STRANDS: SINGLE
 TOPOLOGY: linear
 MOLECULAR TYPE: other nucleic acid, synthetic DNA
 CAGCAGCAGC AGCAG

15

SEQ ID NO: 8

LENGTH: 15

TYPE: nucleic acid

NUMBER OF STRANDS: SINGLE

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid, synthetic DNA

CTGCTGCTGC TGCTG

15

SEQ ID NO: 9

LENGTH: 20

TYPE: nucleic acid

NUMBER OF STRANDS: SINGLE

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid, synthetic DNA

CGCAATTAAC CCTCACTAAAG

20

SEQ ID NO: 10

LENGTH: 21

TYPE: nucleic acid

NUMBER OF STRANDS: SINGLE

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid, synthetic DNA

GCGTAATACG ACTCACTATA G

21

SEQ ID NO: 11

LENGTH: 18

TYPE: nucleic acid

NUMBER OF STRANDS: SINGLE

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid, synthetic DNA

CCAGGTGGCG GACGTGTG

18

SEQ ID NO: 12

LENGTH: 18

TYPE: nucleic acid

NUMBER OF STRANDS: SINGLE

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid, synthetic DNA

CTCCTGCCAA GAGCTCAT

18

SEQ ID NO: 13

LENGTH: 19
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
TGCTGCTCTC CTCGACATG 19

SEQ ID NO: 14
LENGTH: 20
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
GTCATTCCTC CAAGGTCAGC 20

SEQ ID NO: 15
LENGTH: 20
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
CAGTATTCTA GGTAGTGCCC
20

SEQ ID NO: 16
LENGTH: 19
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
AGCAGTGCCC TGAAGATTA 19

SEQ ID NO: 17
LENGTH: 19
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
GCTTGTATAA AGCCTAGCG 19

SEQ ID NO: 18
LENGTH: 20
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
GGAAGGTACA CTTGAATTTC 20

SEQ ID NO: 19
LENGTH: 19
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
AGAAGGCTGC TGGAGCTGG 19

SEQ ID NO: 20
LENGTH: 22
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
CTGTGACAAA TTTTGGTCA AG 22

SEQ ID NO: 21
LENGTH: 20
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
TGCTTGATA AAGCCTAGCG 20

SEQ ID NO: 22
LENGTH: 25
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
CATGGTCCCT TAAATCCATA ACATC 25

SEQ ID NO: 23
LENGTH: 20
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
CATTGGATGT GCATTCTGTG 20

SEQ ID NO: 24
LENGTH: 19
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
GATGTTATGG ATTTAAGGG 19

SEQ ID NO: 25
LENGTH: 19
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
CTTGCGTCTT TCTCTGTGC 19

SEQ ID NO: 26
LENGTH: 20
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
AGCACTGAGC GCGACATGGT 20

SEQ ID NO: 27
LENGTH: 20
TYPE: nucleic acid
NUMBER OF STRANDS: SINGLE
TOPOLOGY: linear
MOLECULAR TYPE: other nucleic acid, synthetic DNA
ACCATGTCGC GCTCAGTGCT 20

SEQ ID NO: 28

LENGTH: 20

TYPE: nucleic acid

NUMBER OF STRANDS: SINGLE

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid, synthetic DNA

TCCTTAAATT CCATAACATC

20

SEQ ID NO: 29

LENGTH: 20

TYPE: nucleic acid

NUMBER OF STRANDS: SINGLE

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid, synthetic DNA

GATGTTATGG AATTTAAGGA

20

[BRIEF DESCRIPTION OF THE DRAWINGS]

Fig. 1 is an electrophoretogram showing the result of RT-PCR.

Fig. 2 is a list showing the comparison of amino acid sequences between human Nap-1 and rat Nap-1.

Fig. 3 is an electrophoretogram showing the distribution of human Nap1 expression in various tissues investigated by Northern blotting.

Fig. 4 is an electrophoretogram showing the results of RT-PCR of Nap1 gene using a human neuronal cell line.

Fig. 5 are photographs showing the results of electrophoresis of cellular DNA after making an antisense PS-ODN be incorporated into the neuronal cell line SH-SY5Y.

[Designation of Document] Abstract

[Abstract]

[Problems of the Invention]

To provide a human Nap1 protein and a DNA encoding said protein.

[Means to Solve the Problems]

A protein having the amino acid sequence represented by SEQ ID NO. 2 or a protein having an amino acid sequence which includes substitution, deletion or addition of one or more amino acids of the amino acid sequence represented by SEQ ID NO. 2 and having apoptosis-suppressing activities, and a DNA encoding the above protein.

[Representative Drawing] None